ChemistryOPEN

Supporting Information

Development of a Wavelength-Shifting Fluorescent Module for the Adenosine Aptamer Using Photostable Cyanine Dyes

Heidi-Kristin Walter, Peggy R. Bohländer, and Hans-Achim Wagenknecht*[a]

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1. Materials and Methods

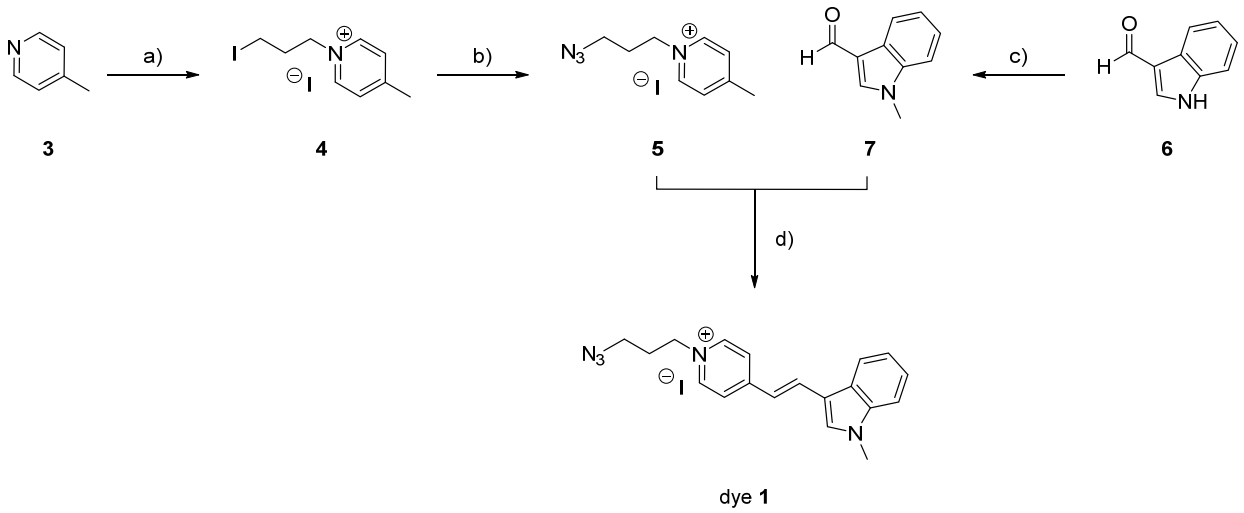
Chemicals and dry solvents were purchased from Aldrich, ABCR, and VWR and were used without further purification unless otherwise stated. Unmodified oligonucleotides werde purchased from Metabion. TLC was performed on Fluka silica gel 60 F254 coated aluminium foil.

The determination of FAB mass spectra was executed by the Institute of Organic Chemistry of the KIT using a Finnigan MAT95 in positive ionization mode. NMR spectra were recorded on a Bruker B-ACS-60, Bruker Avance DRX 400 and a Bruker Avance DRX 500 spectrometer in deuterated solvents (^{1}H at 300, 400 or 500 MHz, ^{13}C at 75, 100 or 125 MHz). Chemical shifts are given in ppm relative to TMS. IR spectra recording were performed by the Institute of Organic Chemistry of the KIT with a Bruker IFS88.

Spectroscopic measurements were recorded in NaP_i -buffer solution (10 mM, pH = 7) with 250 mM NaCl in quartzglass cuvettes (10 mm). Absorption spectra were recorded with a Perkin Elmer Lambda 750 UV/vis spectrometer equipped with at 20 °C. Fluorescence was measured with a Horiba Scientific FluoroMax-4 spectrofluorometer with a step width of 1 nm and an integration time of 0.2 s. All spectra were recorded at 20 °C with excitation and emission bandpass of 3 nm and are corrected for Raman emission from the buffer solution. Quantum yields were determined with Quantaurus QY C11347 of Hamamatsu.

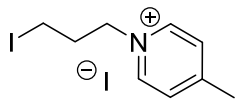
DNA1-**DNA22** were purified with a Reversed Phase Supelcosil™ LC-C18 column (250 x 10 mm, 5 µm) on a Shimadzu HPLC sytem (autosampler, SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A). Purification was confirmed by MS (MALDI-TOF) either on a Bifelx-IV spectrometer from Bruker Daltonics or Autoflex-III Smartbeam from Bruker Daltonics in the linear negative mode (matrix: either 2:1 mixture of 2,4,6-trihydroxyacetophenone (0.3 M in EtOH) and diammoniumcitrate (0.1 M in H_2O) or 1:9 mixture of diammoniumhydrogencitrate (100 g/L) and a saturated 3-hydroxypicolinic acid solution (10 g/L in 50% acetonitrile in water)). DNA concentrations were measured by their absorbance in water at 260 nm on a ND-1000 spectrometer from NanoDrop in the nucleic acid mode.

2. Synthesis of dye 1



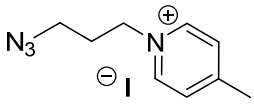
Scheme S1: Synthesis of dye **1**; a) 1,3-diiodopropane, MeCN, reflux, 16 h; b) NaN_3, MeCN, reflux, 16 h; c) K_2CO_3, dimethyl carbonate, DMF, 130 °C, 19 h; d) piperidine, EtOH, 80 °C, 4 h.

Synthesis of 4:



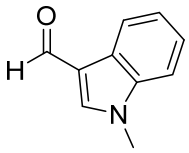
Commercially available 4-picoline (**3**) (466 mg, 5.00 mmol) and 1,3-diiodopropane (5.91 g, 20.0 mmol) were dissolved in 10 mL acetonitrile and refluxed for 16 hours. After cooling to room temperature the solvent was removed under reduced pressure. To the remaining oil ethyl acetate was added and the mixture was treated in the ultrasonic bath. The forming precipitate was collected by filtration, washed several times with ethyl acetate and dried. 1.83 g (94 %) of a slightly yellow powder was obtained. Spectral data was in accordance with the literature.[1]

Synthesis of 5



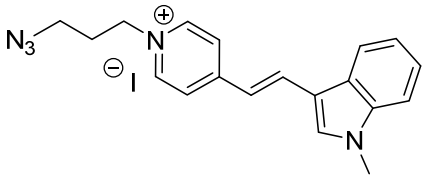
1-(3-Iodopropyl)-4-methylpyridinium iodide (4) (900 mg, 2.31 mmol) were dissolved in 12 mL acetonitrile together with sodium azide (376 mg, 5.78 mmol) and refluxed for 16 h. After cooling to room temperature the solvent was removed under reduced pressure. To the residue 15 mL dichloromethane was added and the resulting solid was filtered off. The solvent was removed *in vacuo* to yield 611 mg (87 %) of a brown oil. Spectral data was in accordance with the literature.[1]

Synthesis of 7



Under argon atmosphere indole-3-carbaldehyde (**6**) (1.45 g, 10.0 mmol), potassium carbonate (1.52 g, 11.0 mmol) and dimethyl carbonate (2.70 g, 30.0 mmol) were dissolved in 10 mL dry DMF. The reaction mixture was then stirred at 130 °C for 19 h. After cooling to room temperature the reaction mixture was poured on ice. The aqueous layer was extracted 3 times with 150 mL ethyl acetate. The combined organic layers were washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford 1.41 g (89 %) of light brown solid. Spectral data was in accordance with the literature.[2]

Synthesis of 1



Under argon **5** (90 mg, 0.30 mmol) and **7** (48 mg, 0.30 mmol) were dissolved in 4 mL EtOH and piperidine (0.07 mL, 0.73 mmol) were added. The reaction mixture was then refluxed for

4 h. After cooling to room temperature the resulting precipitate was collected by filtration and washed with diethyl ether (3 times). Diethyl ether was added to the supernatant and the precipitated was filtered off and washed with diethyl ether (3 times). 108 mg (80 %) of a black-red solid was obtained.

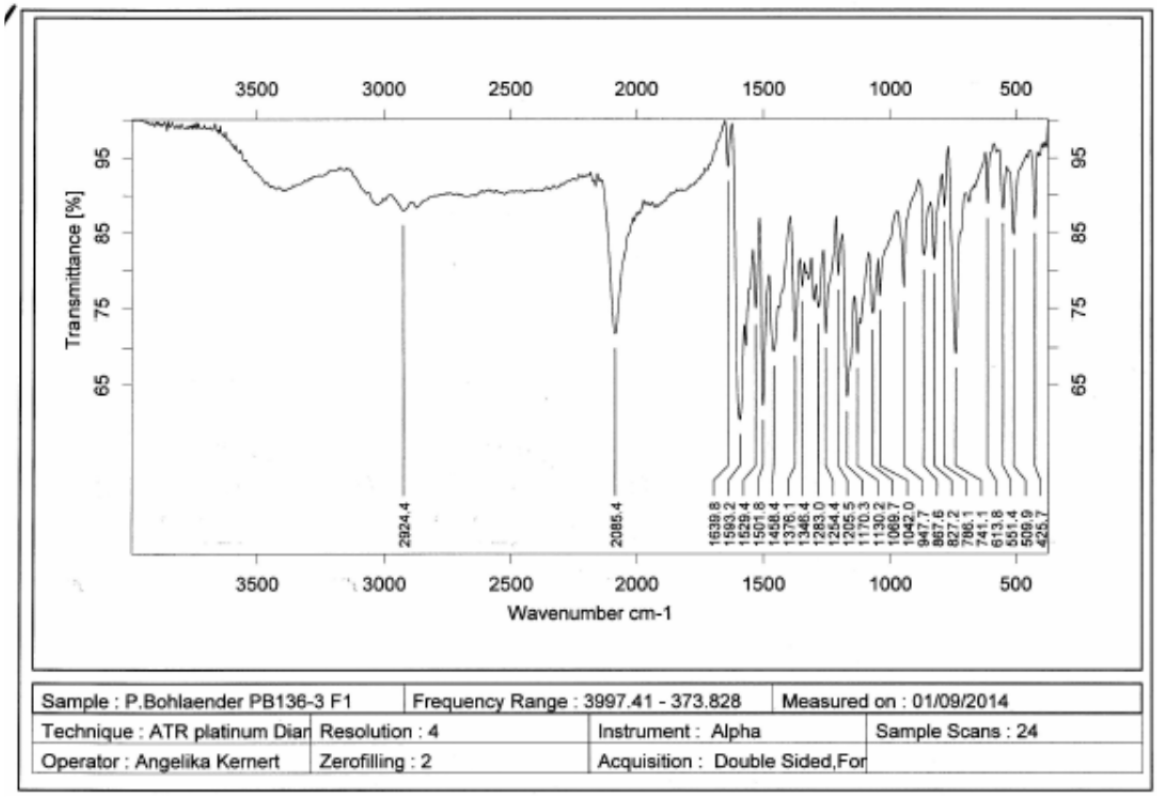
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.27.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 2924 (w), 2085 (s), 1593 (s), 1376 (w), 1170 (m).

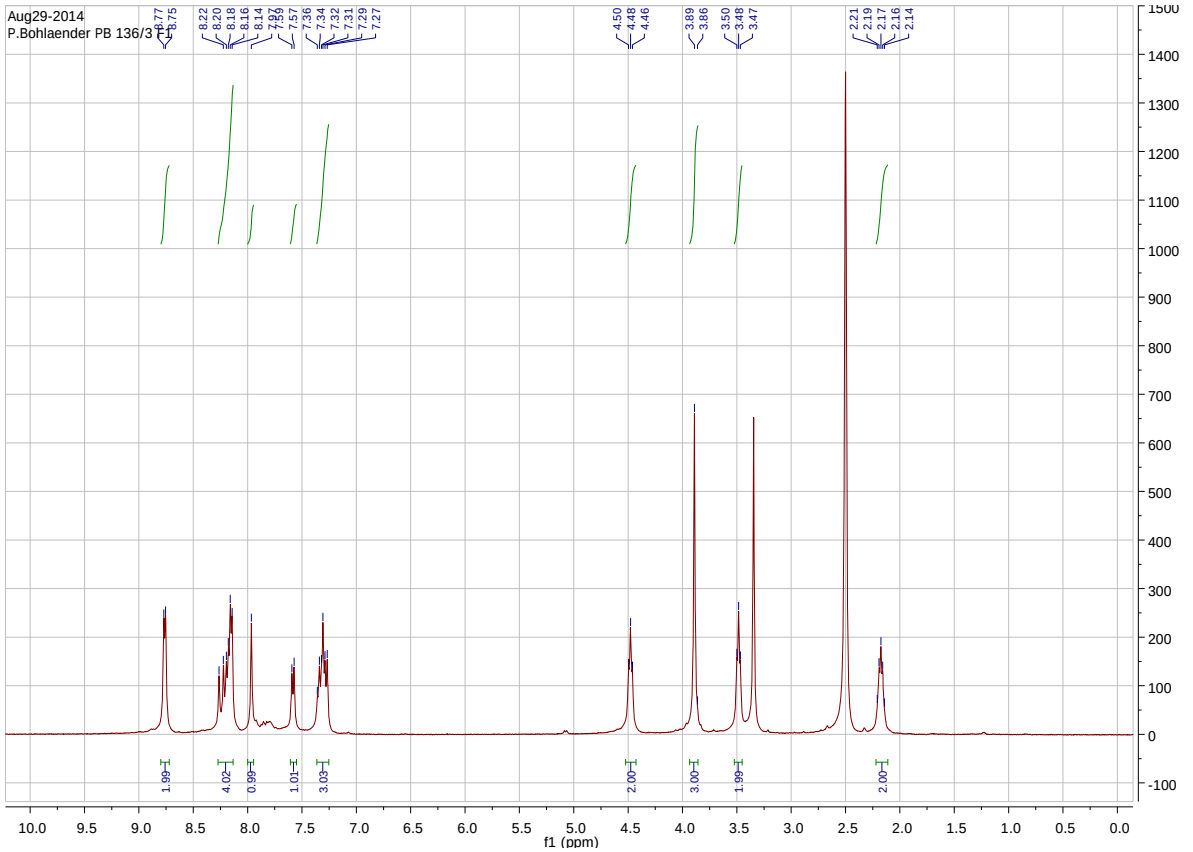
^{1}H-NMR (400MHz; DMSO-d_6):
δ (ppm) = 2.18 (p, *J* = 6.7, 2H), 3.48 (t, *J* = 6.5, 2H), 3.89 (s, 3H), 4.48 (t, *J* = 7.1, 2H), 7.25 – 7.36 (m, 3H), 7.58 (d, *J* = 8.1, 1H), 7.97 (s, 1H), 8.13 – 8.27 (m, 4H), 8.76 (d, *J* = 6.7, 2H).

^{13}C-NMR (100 MHz, DMSO-d_6):
δ (ppm) = 29.5, 33.1, 47.6, 56.7, 111.0, 112.6, 116.8, 120.5, 121.4, 121.9, 123.0, 125.3, 135.8, 136.0, 138.0, 143.4, 154.6.

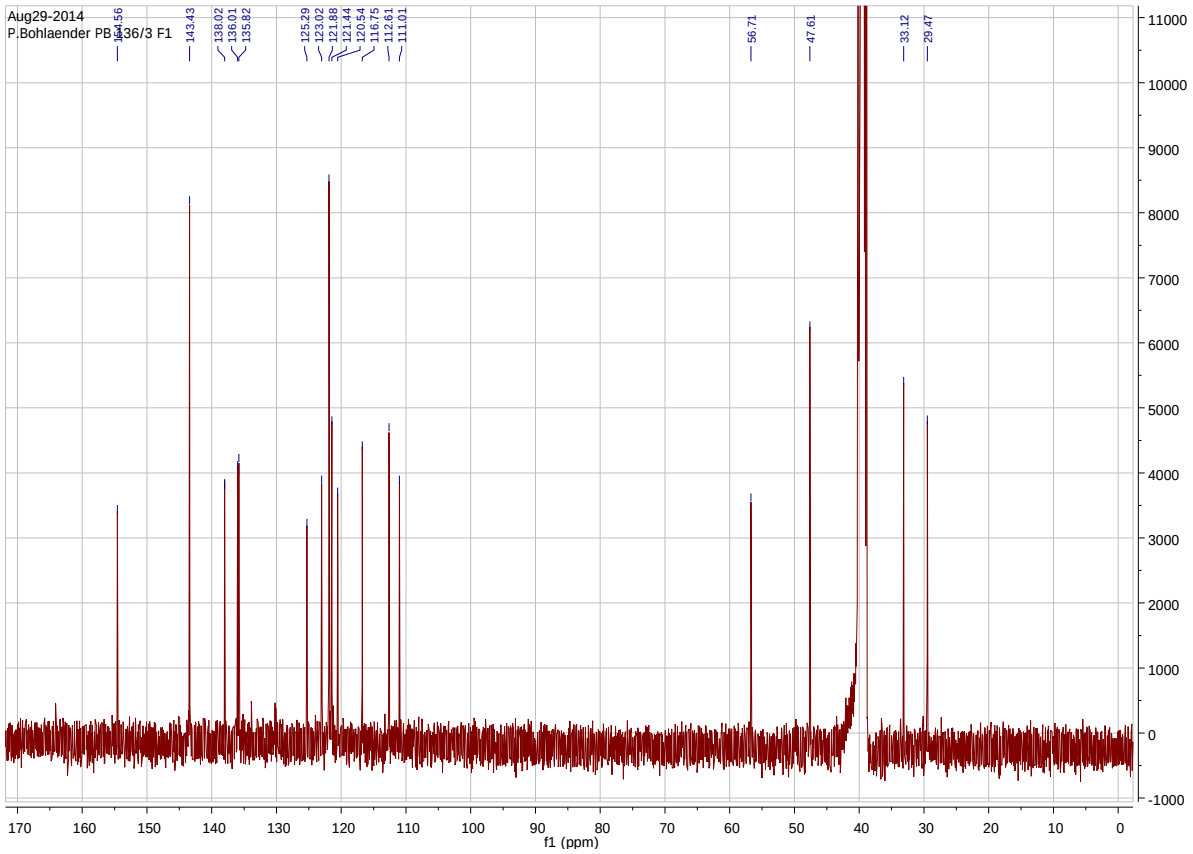
MS (FAB) m/z (%): 318.2 (100) [M^+].
HR-MS (FAB) m/z: calculated for $C_{19}H_{20}N_5^+$ [M^+]: 318.1713, found: 318.1715.



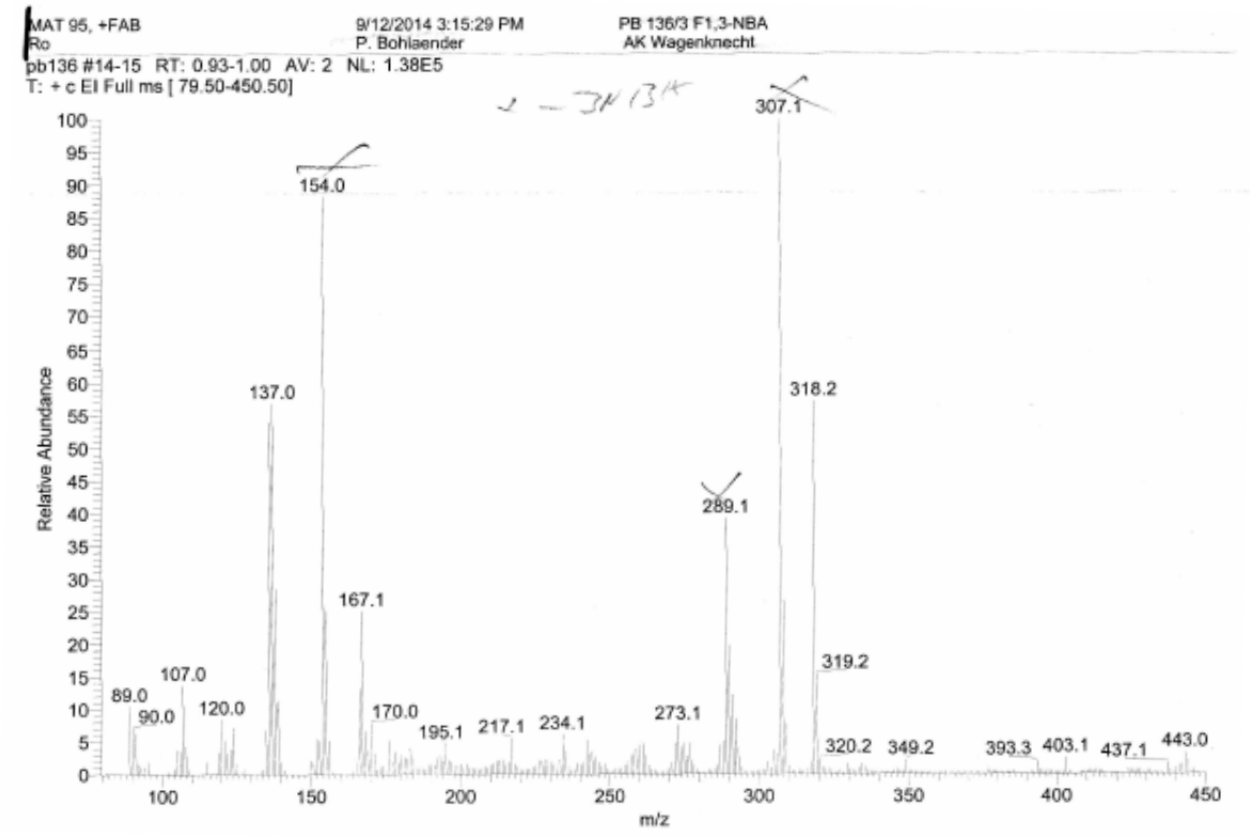
Scheme S2: IR of azide **1**.



Scheme S3: ^{1}H-NMR of azide **1**.



Scheme S4: ^{13}C-NMR of azide **1**.



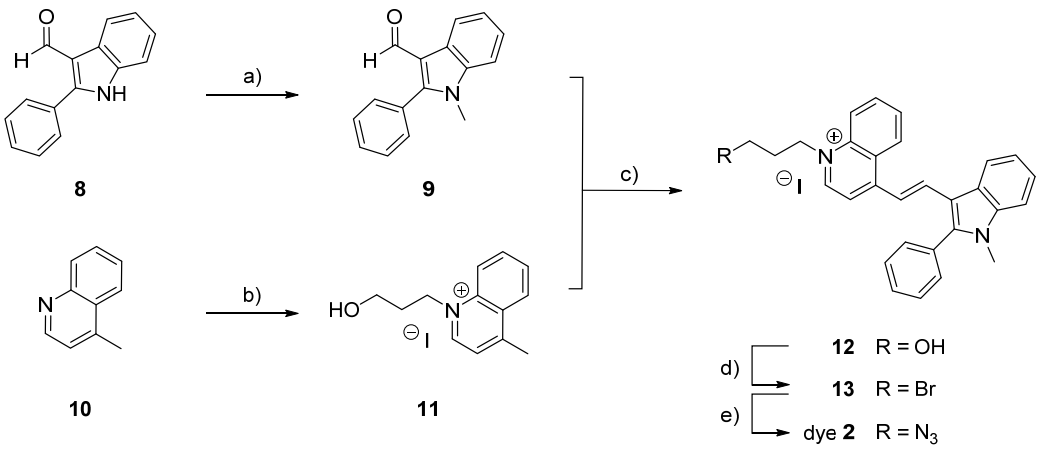
Scheme S5: MS (FAB) of azide **1**.

pb136-c4#11 RT: 0.74
T: + c EI Full ms [79.48-450.48]
m/z= 318.0687-318.3179

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
318.1715	3062.0	100.00	318.1713	0.13	$C_{19}H_{20}N_5$

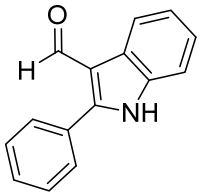
Scheme S6: HR-MS (FAB) of azide **1**.

3. Synthesis of dye 2



Scheme S7: Synthesis of dye **2**; a) K_2CO_3, dimethyl carbonate, DMF, 130 °C, 19 h; b) 3-iodopropanole, 1,4-dioxane, reflux, 2 h; c) piperidine, EtOH, 80 °C, 19 h; d) PPh_3, CBr_4, DCM, rt, 2 h, e) NaN_3, NaI, DMF, rt, 19 h.

Synthesis of 9



Under argon, a mixture of 2-phenyl-1H-indole-3-carbaldehyde (**8**) (2.21 g, 10.0 mmol), K_2CO_3 (1.52 g, 11.1 mmol) and dimethylcarbonate (3.60 g, 3.37 mL, 40.0 mmol) in 10 mL dimethylformamide was stirred at 130 °C for 19 h. After cooling to room temperature the mixture was poured on 100 g ice. The aqueous phase was extracted four times with 100 mL ethyl acetate. The organic phase was washed two times with 150 mL water, dried with Na_2SO_4 and the solvent was removed at 50 °C and reduced pressure. The product crystallized out of the residual yellow. Drying under reduced pressure yields a yellow solid (98 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.80.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3044 (w), 1642 (s), 1379 (m), 1069 (m).

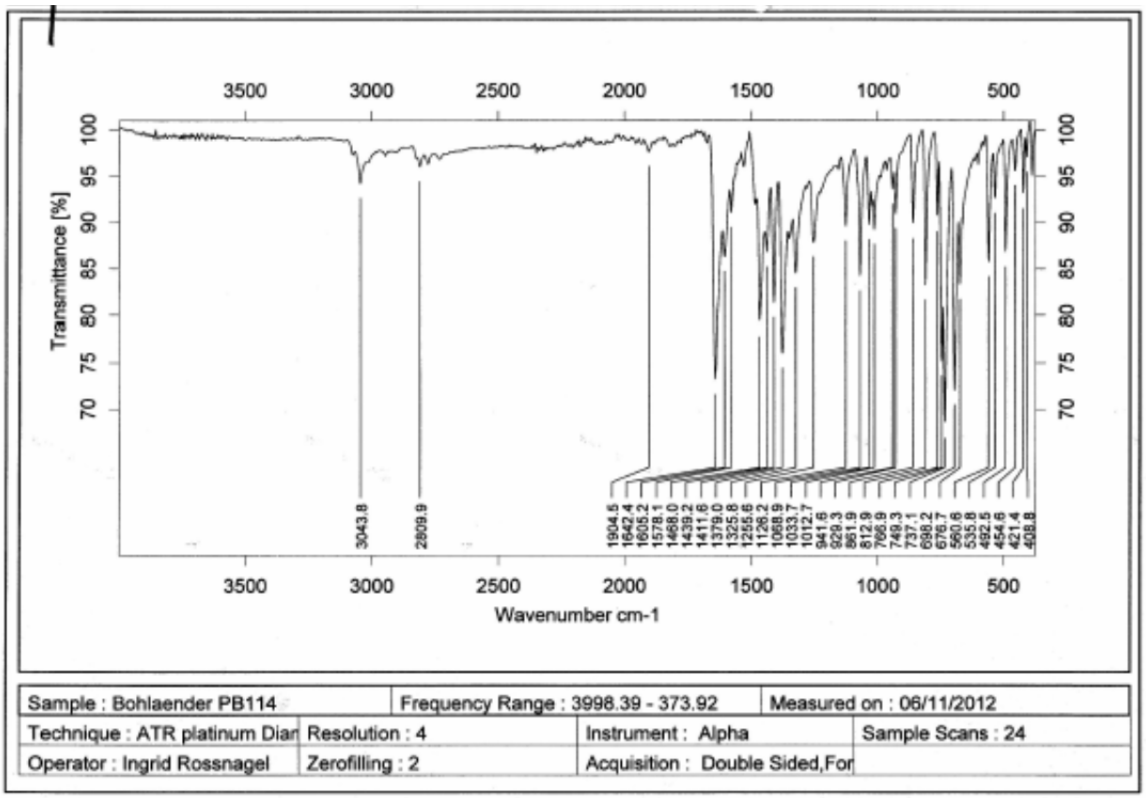
^{1}H-NMR (300MHz; DMSO-d_6):

δ (ppm) = 3.69 (s, 3H), 7.28 – 7.42 (m, 2H), 7.65 (dp, *J* = 10.0, 3.4, 2.9, 6H), 8.16 – 8.31 (m, 1H), 9.61 (s, 1H).

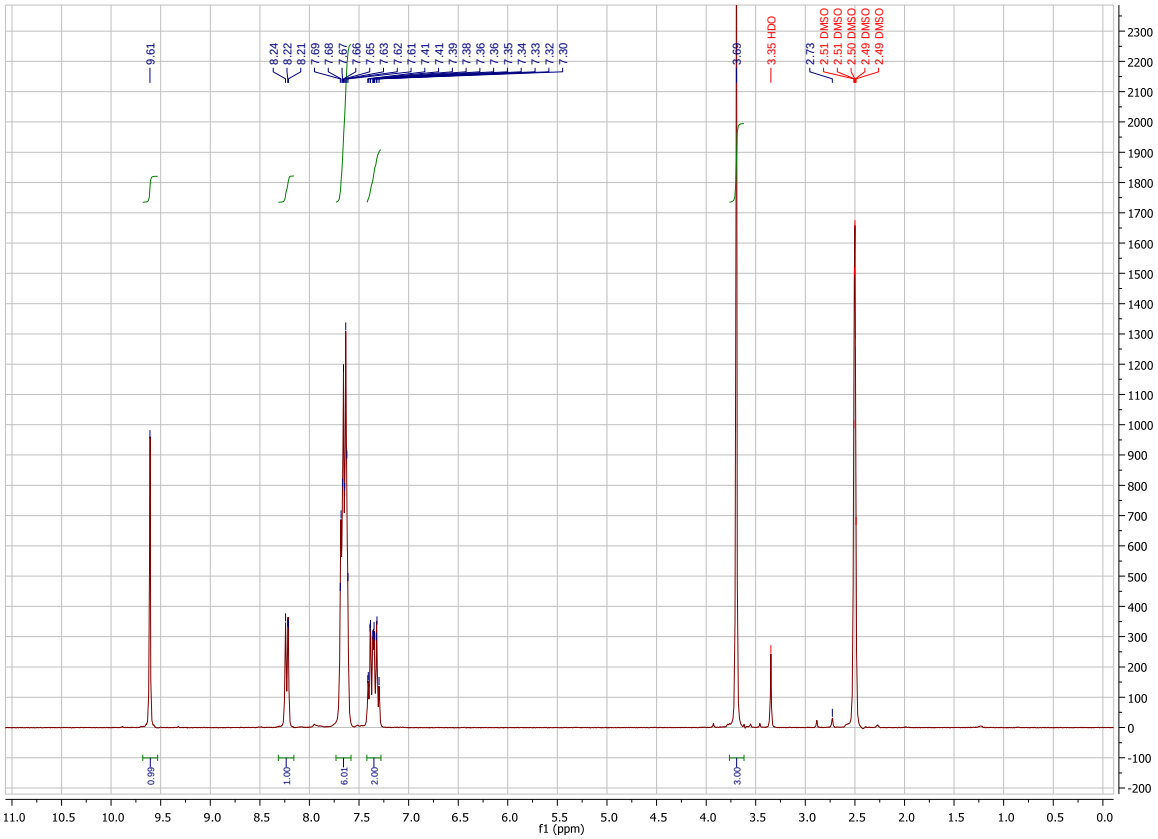
^{13}C-NMR (75 MHz, DMSO-d_6):

δ (ppm) = 31.0, 111.0, 114.4, 120.8, 122.9, 123.7, 124.5, 128.1, 128.6, 129.9, 131.0, 137.1, 151.2, 185.2.

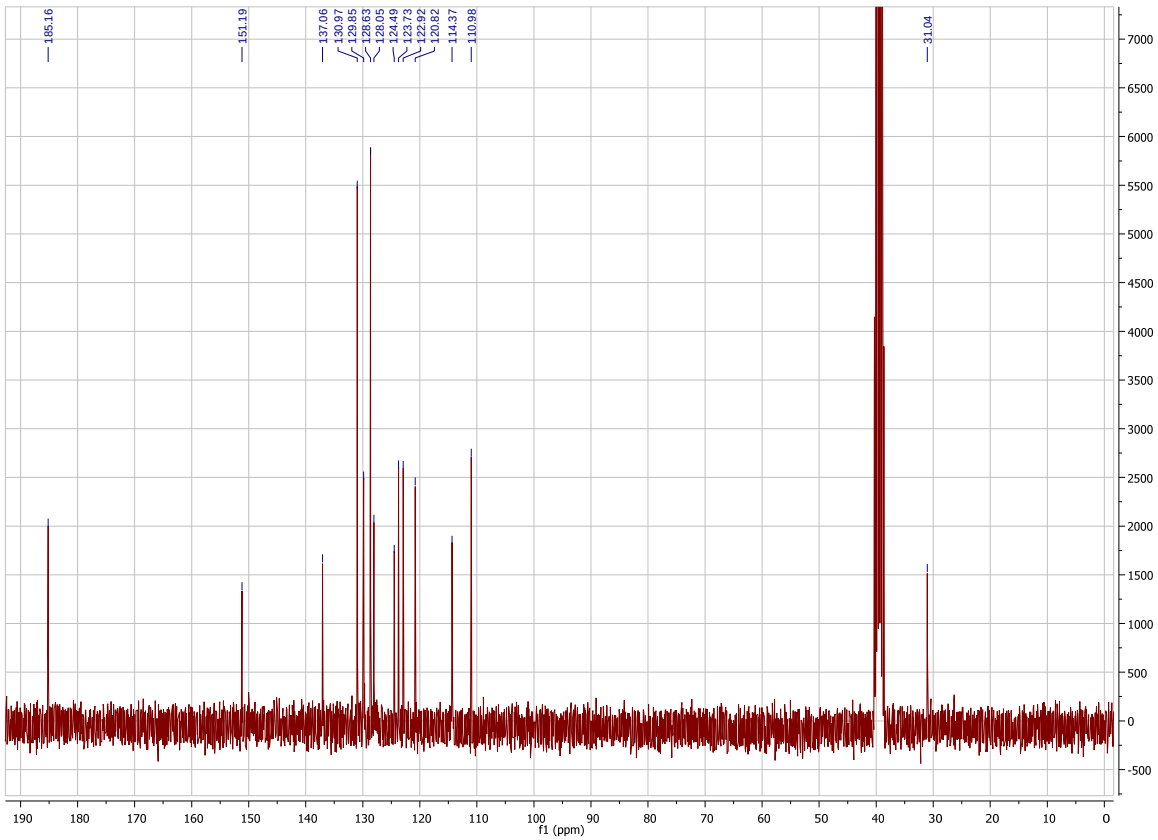
MS (FAB) m/z (%): 236.4 (100) [M^+].



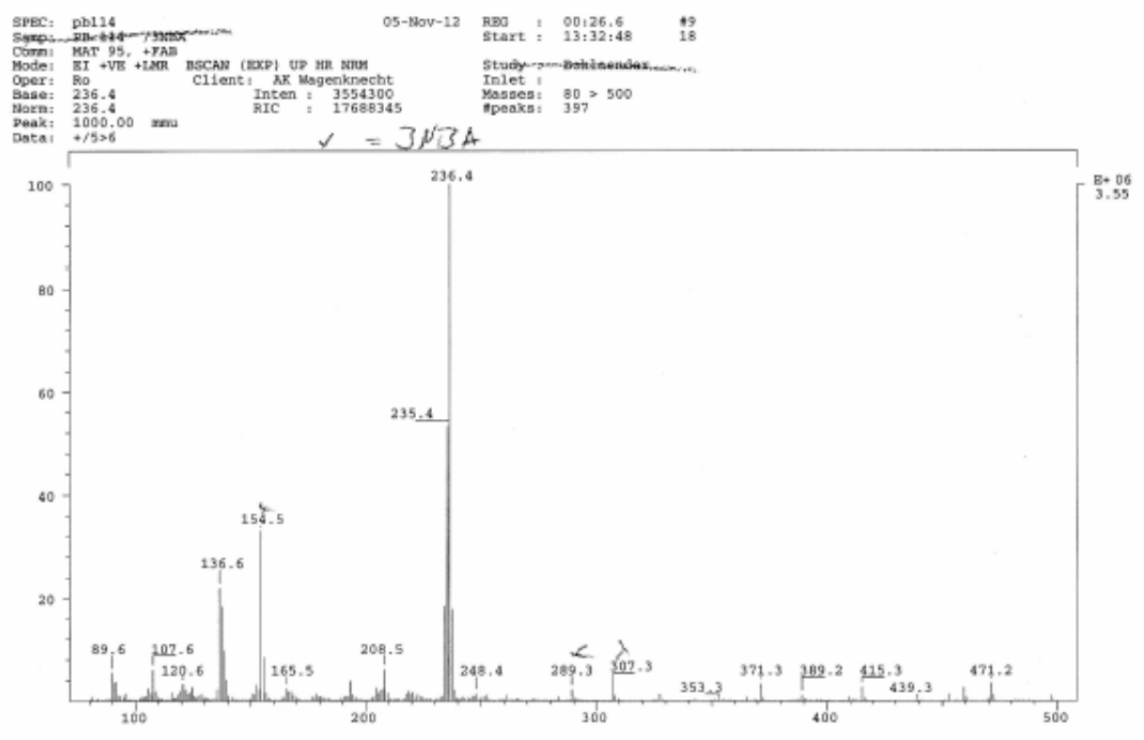
Scheme S8: IR of compound **9**.



Scheme S9: ^{1}H-NMR of compound **9**.

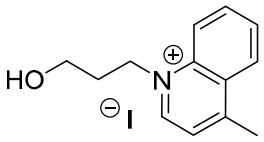


Scheme S10: ^{13}C-NMR of compound **9**.



Scheme S11: MS (FAB) of compound **9**.

Synthesis of 11



Under argon, a mixture of 4-methylquinoline (**10**) (0.72 g, 0.67 mL, 5.00 mmol) and 3-iodo-1-propanol* (0.72 mL, 1.40 g, 7.50 mmol) in 3 mL 1,4-dioxane was stirred in a headspace vial at 101 °C for 2 h. After cooling to room temperature 5 mL diethyl ether were added and after precipitation the product was collected and washed three times with diethyl ether. Drying under reduced pressure yields a yellow solid (95 %).

* Please note: It is crucial to use fresh 3-iodo-1-propanol (e.g. via Finkelstein-reaction of 3-chloro-1-propanol and NaI in acetone).

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3351 (s), 2934 (m), 2867 (m), 1366 (w), 1060 (m).

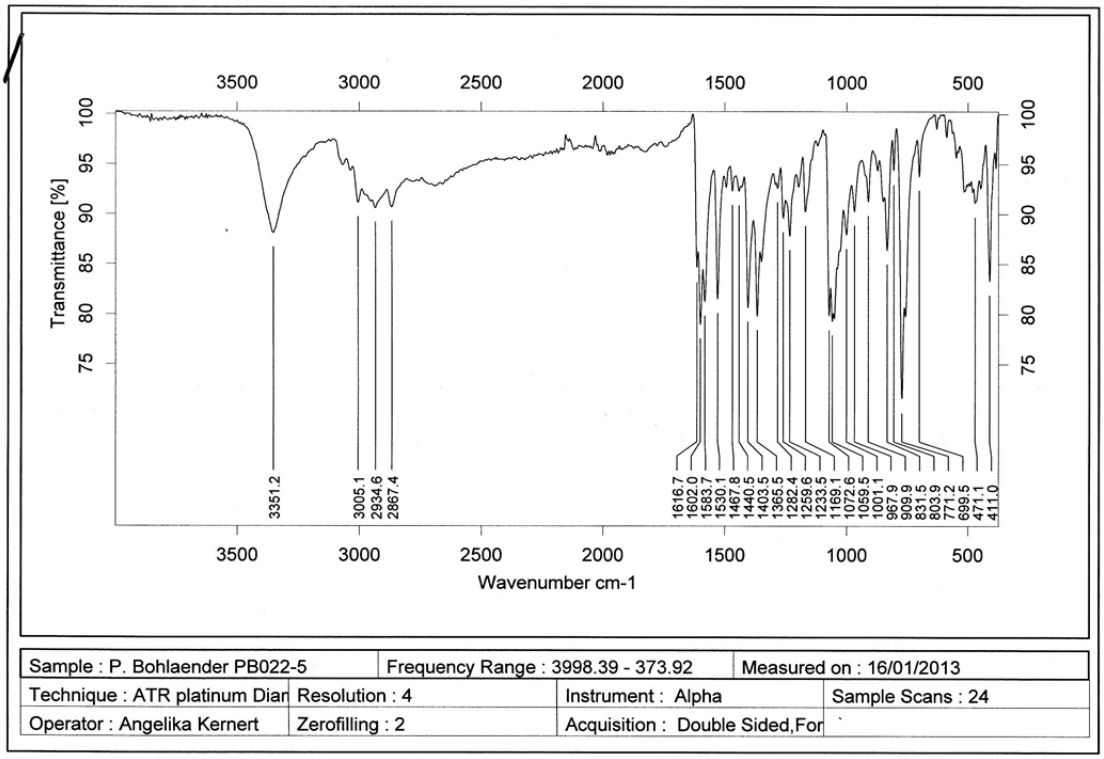
^{1}H-NMR (300MHz; DMSO-d_6):

δ (ppm) = 2.11 (t, *J* = 6.5, 2H), 3.00 (s, 3H), 3.52 (t, *J* = 5.7, 2H), 4.56 (s, 1H), 5.07 (t, *J* = 7.2, 2H), 8.01 – 8.09 (m, 2H), 8.21 – 8.31 (m, 1H), 8.50 – 8.61 (m, 2H), 9.39 (d, *J* = 6.0, 1H).

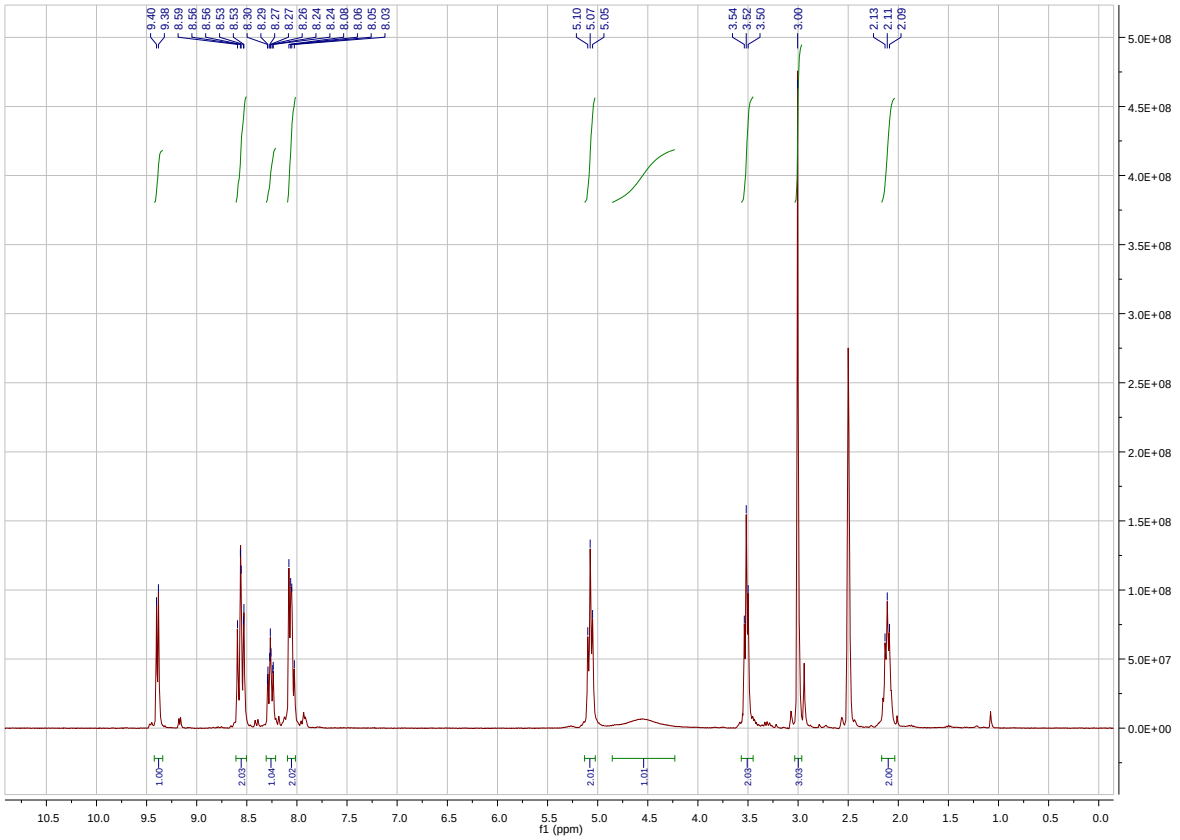
^{13}C-NMR (75 MHz, DMSO-d_6):

δ (ppm) = 19.7, 32.0, 54.8, 57.4, 119.3, 122.6, 127.2, 128.9, 129.5, 135.0, 136.7, 148.7, 158.5.

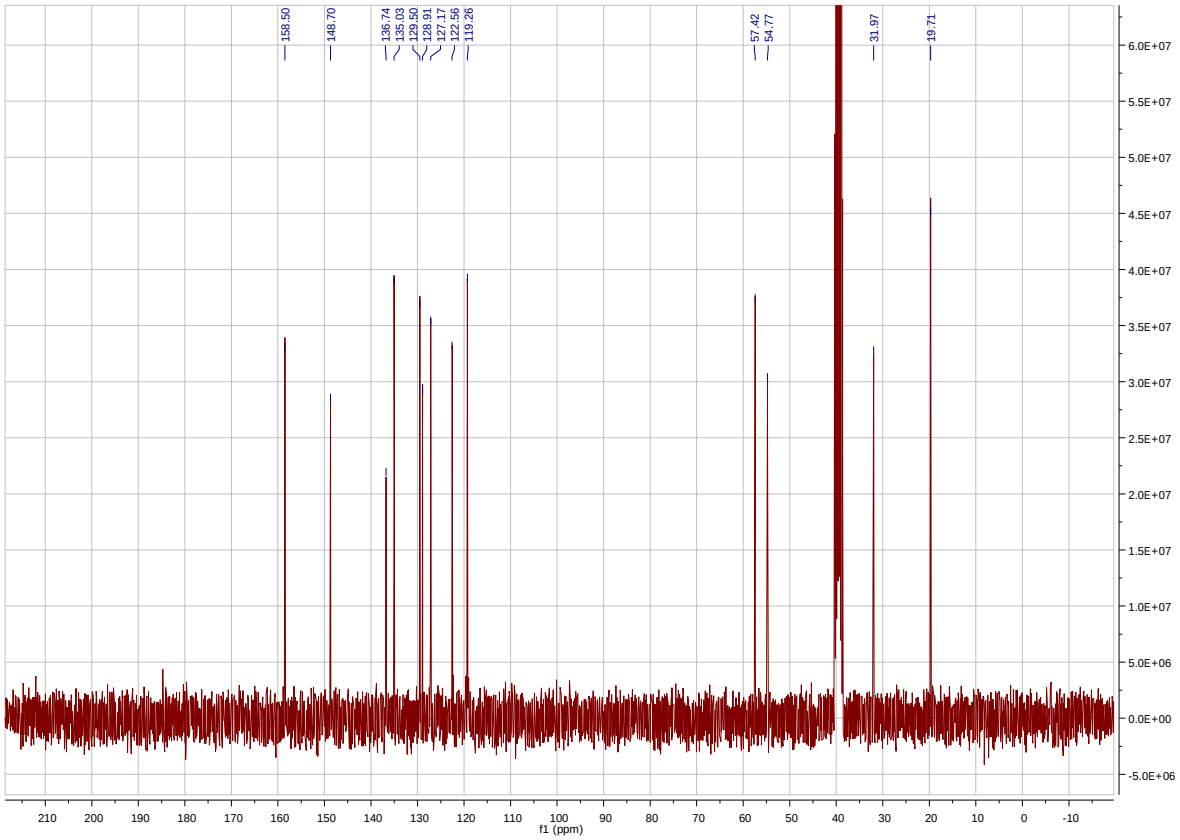
MS (FAB) m/z (%): 202.3 (100) [M].



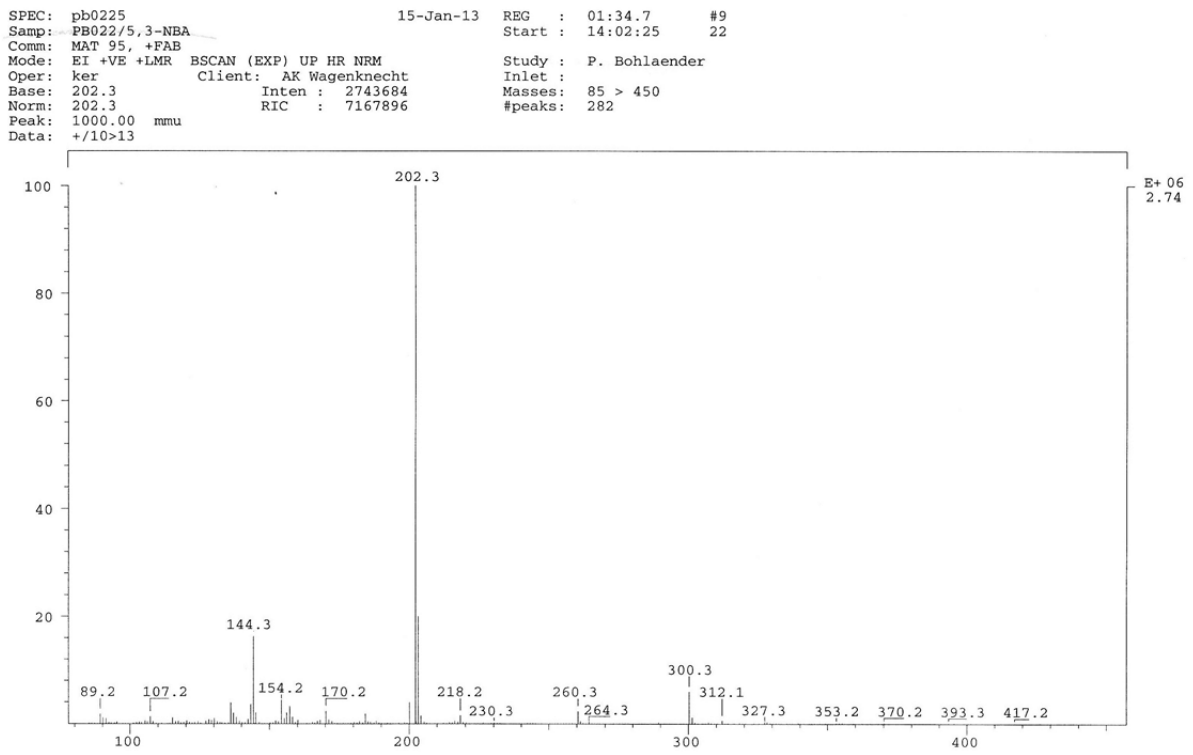
Scheme S12: IR of compound **11**.



Scheme S13: ^{1}H-NMR of compound **11**.

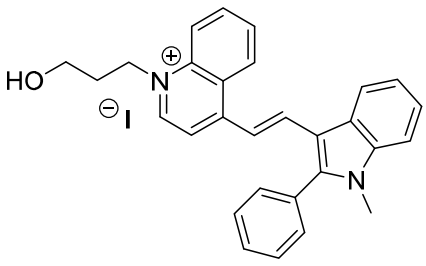


Scheme S14: ^{13}C-NMR of compound **11**.



Scheme S15: MS (FAB) of compound **11**.

Synthesis of 12



Under argon, to a mixture of compound **11** (0.33 g, 1.00 mmol) and compound **9** (0.47 g, 2.00 mmol) in 13 mL ethanol, piperidine (0.22 mL, 0.19 g, 2.20 mmol) was added and the reaction mixture was stirred in a headspace vial at 80 °C for 19 h. After cooling to room temperature the precipitated product was collected and washed three times with diethyl ether. A 2nd product fraction could be achieved from mother liquor. Drying under reduced pressure yields a dark-red solid (83 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.29.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3355 (w), 1580 (m), 1550 (s), 1386 (m), 1227 (s).

^{1}H-NMR (300MHz; DMSO-d_6):

δ (ppm) = 2.06 (p, *J* = 6.5, 2H), 3.50 (q, *J* = 5.4, 2H), 3.71 (s, 3H), 4.79 (t, *J* = 4.8, 1H), 4.90 (t, *J* = 7.1, 2H), 7.37 – 7.48 (m, 2H), 7.59 – 7.74 (m, 6H), 7.80 – 7.99 (m, 4H), 8.18 (t, *J* = 7.9, 1H), 8.39 (t, *J* = 7.6, 2H), 8.68 (d, *J* = 8.4, 1H), 8.98 (d, *J* = 6.6, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):

δ (ppm) = 31.5, 31.8, 53.7, 57.4, 111.3, 111.9, 113.6, 113.7, 118.8, 121.0, 122.5, 123.7, 124.7, 125.7, 126.2, 128.6, 129.0, 129.3, 129.8, 131.0, 134.7, 137.7, 137.9, 146.6, 147.2, 153.5, 162.4.

MS (FAB) m/z (%): 419.1 (100) [M^+].

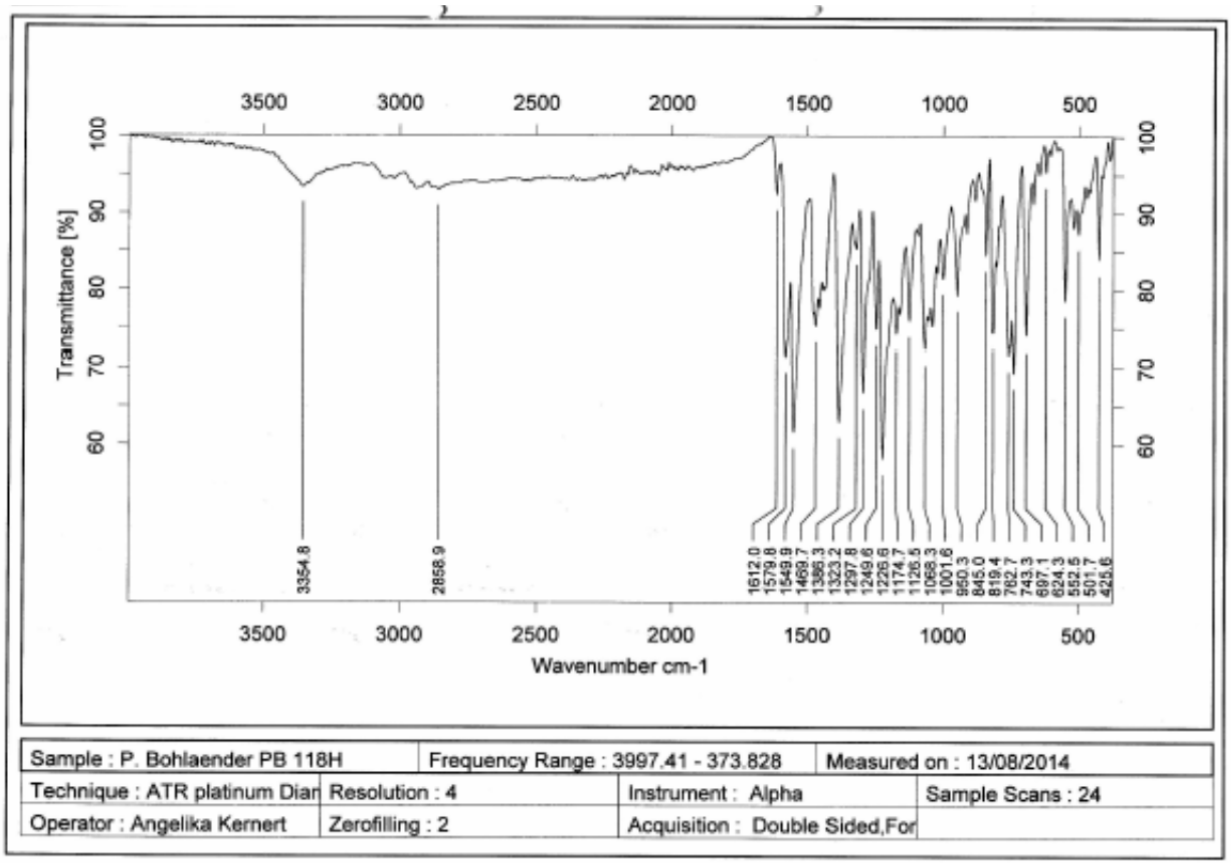
HR-MS (FAB) m/z: calculated for $C_{29}H_{27}N_2O$ [M^+]: 419.2123, found: 419.2121.

Elementary analysis

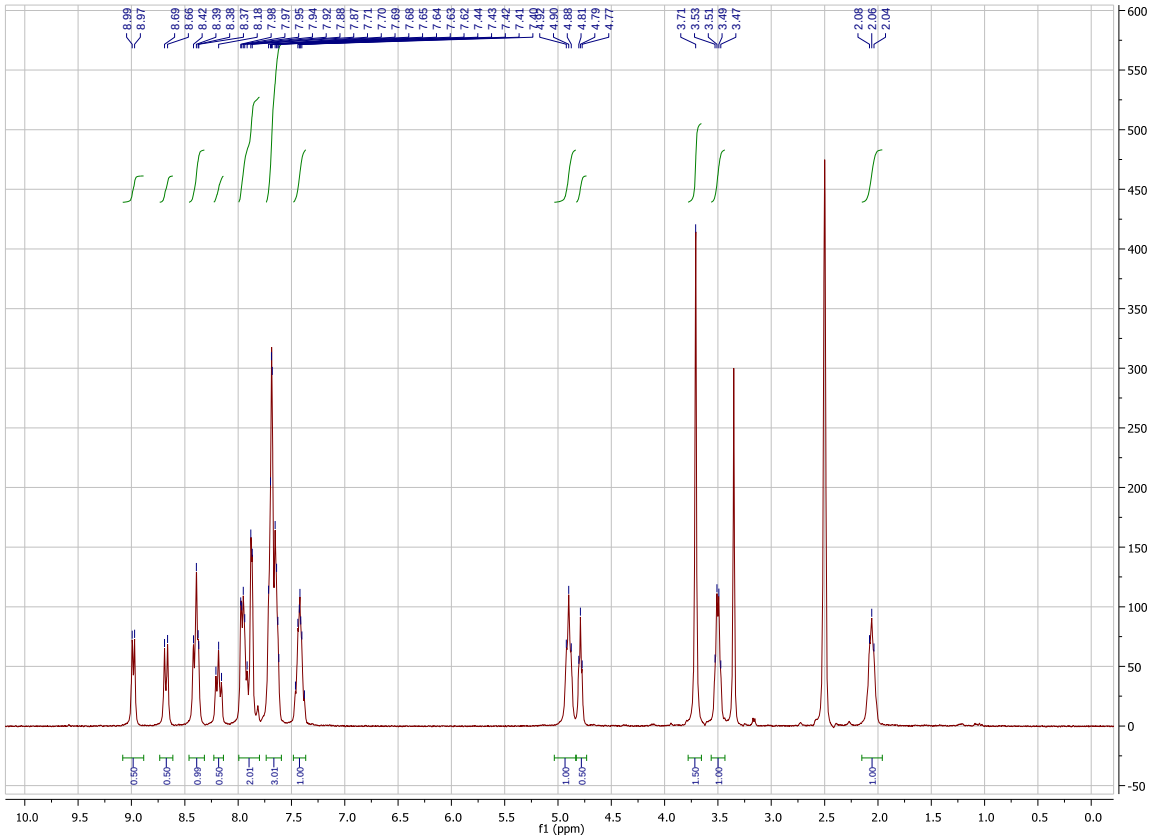
calculated for $C_{29}H_{27}IN_2O$:

N: 5.13 % → found: 5.01 % C: 63.74 % → found: 62.89 %

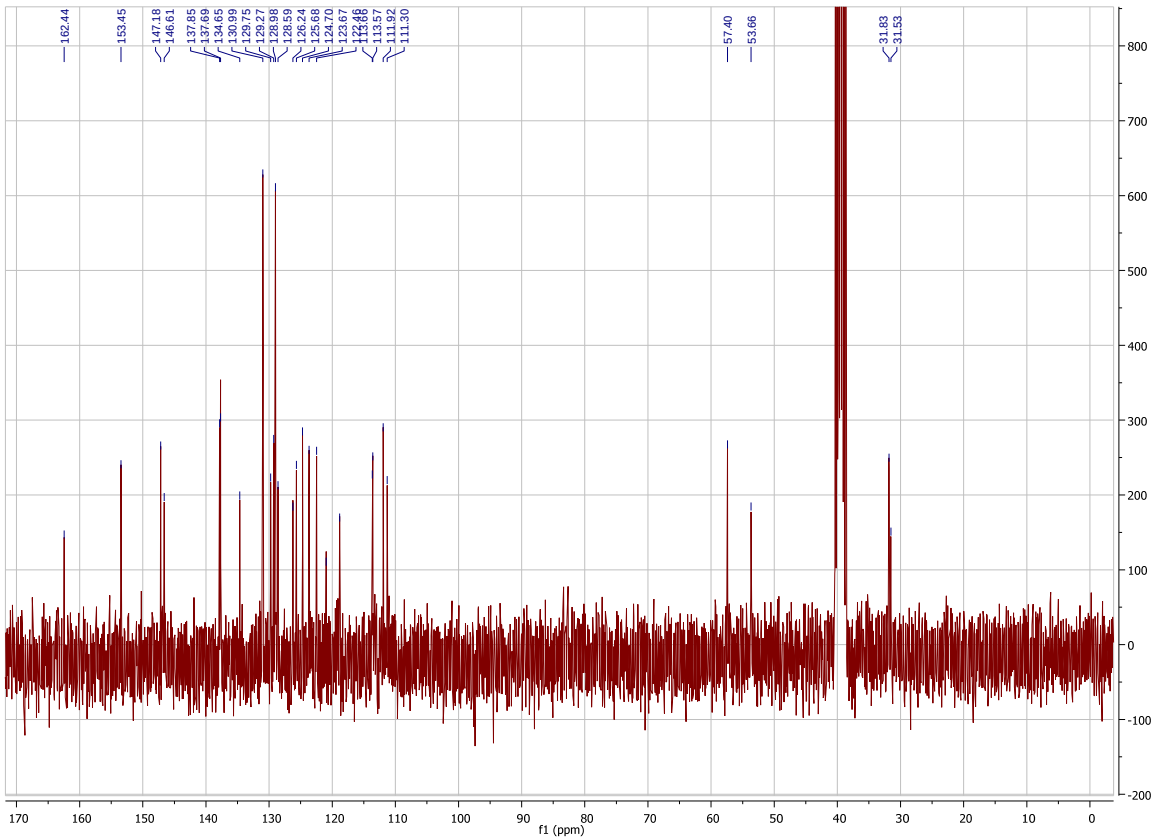
H: 4.98 % → found: 4.95 %



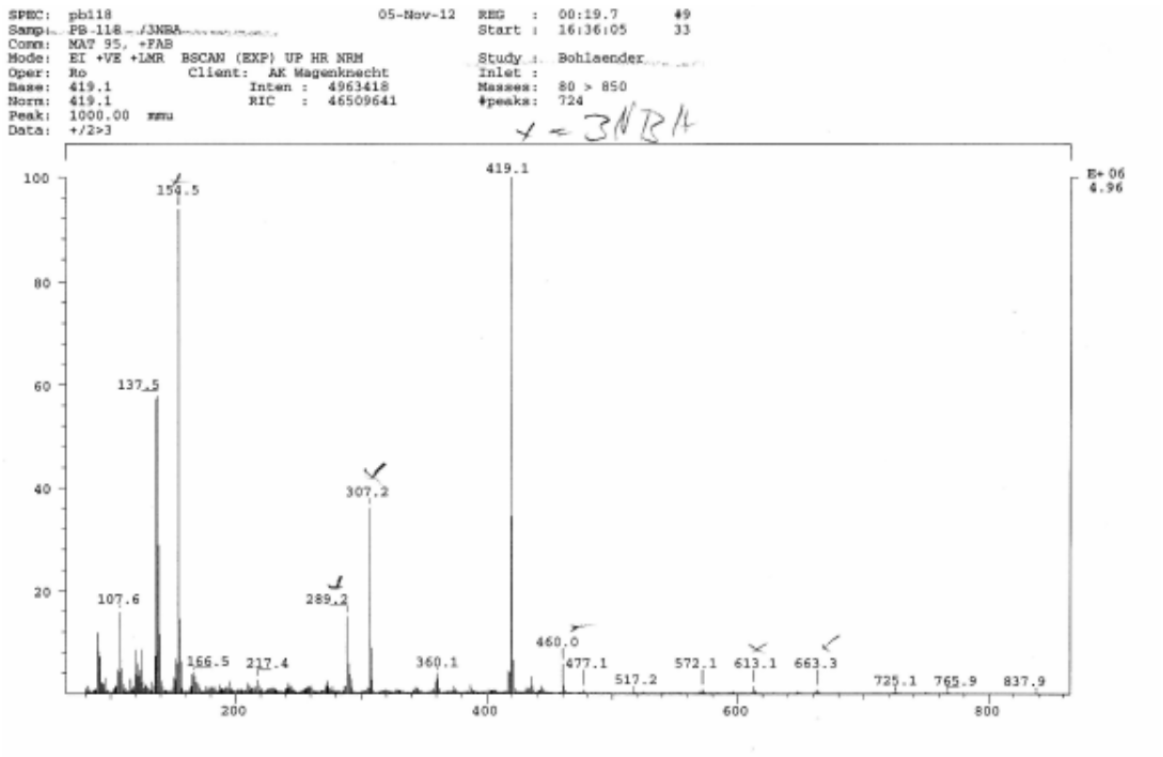
Scheme S16: IR of compound **12**.



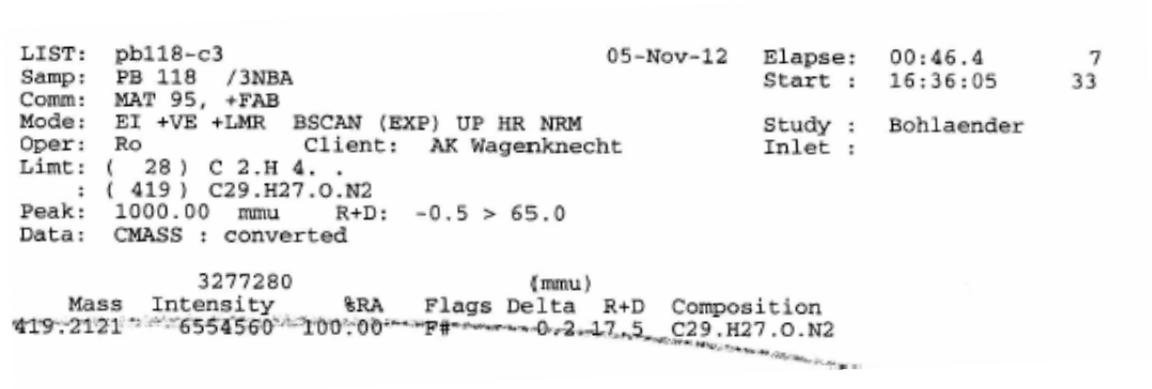
Scheme S17: ^{1}H-NMR of compound **12**.



Scheme S18: ^{13}C-NMR of compound **12**.



Scheme S19: MS (FAB) of compound **12**.



Scheme S20: HR-MS (FAB) of compound **12**.

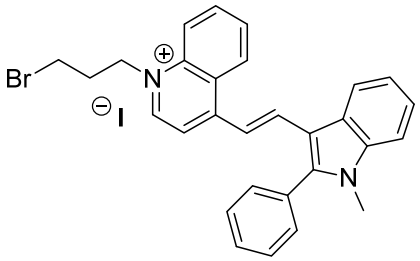
Berechnet: N: 5,13 % C: 80,14 % H: 6,26 % S: C: 4,93 % I: 22,66 %

Gefunden: N: 5,01 C: 62,60 H: 4,94 S:

Gefunden: N: 4,92 C: 62,89 H: 4,95 S:

Scheme S21: Elementary analysis of compound **12**.

Synthesis of 13



Under argon, a mixture of **12** (0.27 g, 0.50 mmol), triphenylphosphine (0.39 g, 1.50 mmol) and tetrabromomethane (0.55 g, 1.65 mmol) in 5 mL dichloromethane was stirred in a headspace vial at room temperature for 2 h. After addition of 0.1 g NaBr to the mixture it was solubilized in 75 mL acetone and 10 mL methanol and the solvent was removed at 50 °C and reduced pressure reduced to a residual volume of 10 mL. The suspension was diluted with 5 mL methanol and the product was crystallized by use of ultra sonic bath. The precipitation was collected and washed three times with diethylether. Drying under reduced pressure yields a dark-red solid (90 %).

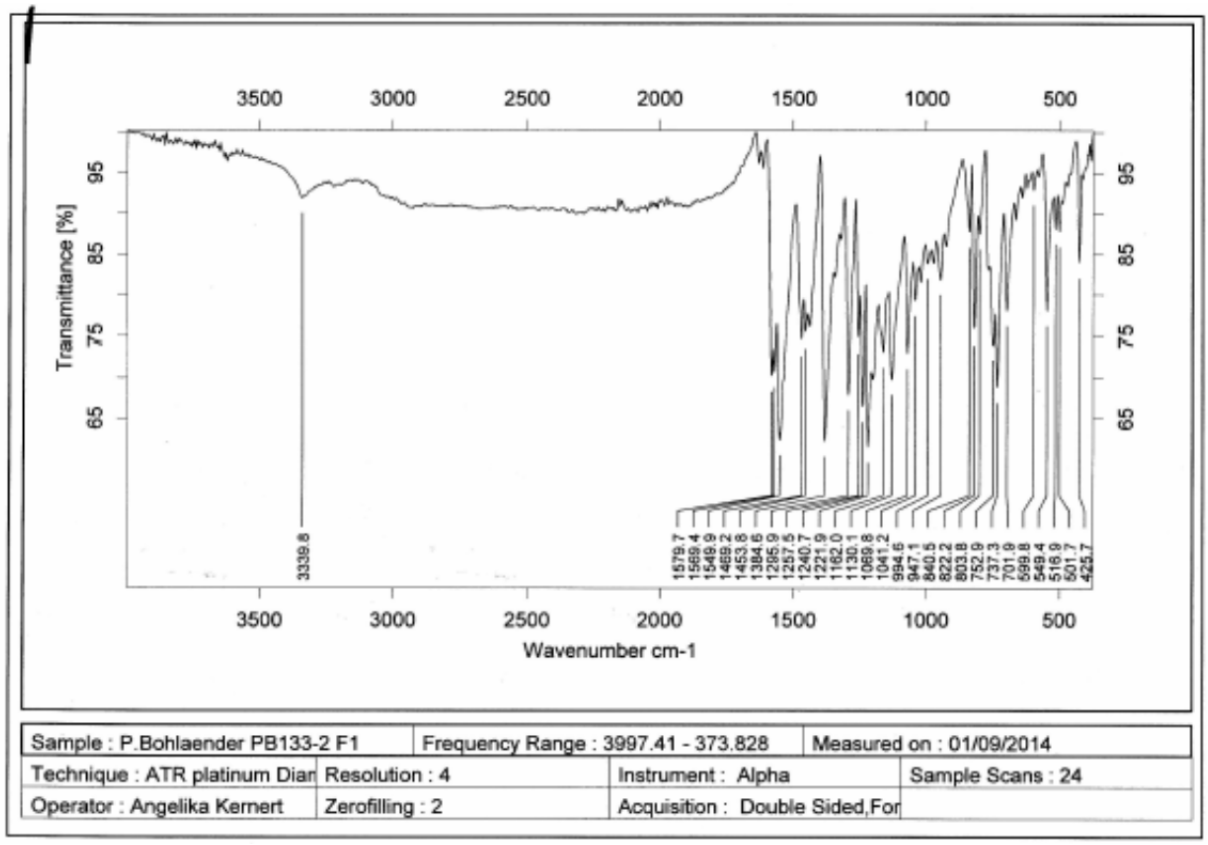
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.48.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3340 (w), 1550 (s), 1385 (s), 1222 (m), 1130 (w).

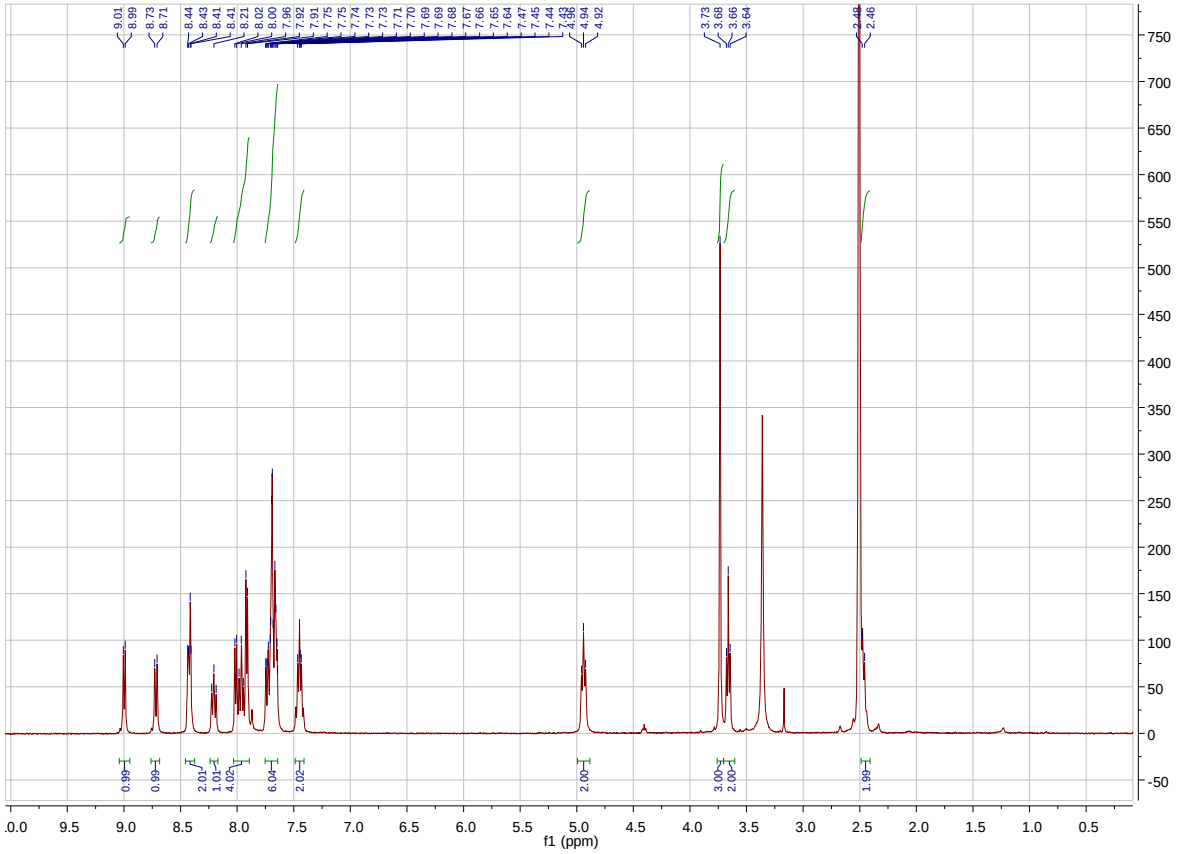
^{1}H-NMR (400MHz; DMSO-d_6):
δ (ppm) = 2.41 – 2.49 (m, 2H), 3.66 (t, *J* = 6.9, 2H), 3.74 (s, 3H), 4.88 – 4.99 (m, 2H), 7.41 – 7.49 (m, 2H), 7.64 – 7.75 (m, 6H), 7.89 – 8.03 (m, 4H), 8.17 – 8.24 (m, 1H), 8.44 (dd, *J* = 8.0, 4.6, 2H), 8.72 (d, *J* = 9.0, 1H), 8.95 – 9.04 (m, 1H).

^{13}C-NMR (100 MHz, DMSO-d_6):
δ (ppm) = 30.5, 31.5, 31.8, 54.5, 111.3, 111.9, 113.5, 113.8, 118.6, 120.9, 122.4, 123.6, 124.6, 125.7, 126.3, 128.5, 128.9, 129.2, 129.7, 130.9, 134.7, 137.7, 137.8, 137.9, 146.5, 147.3, 153.7.

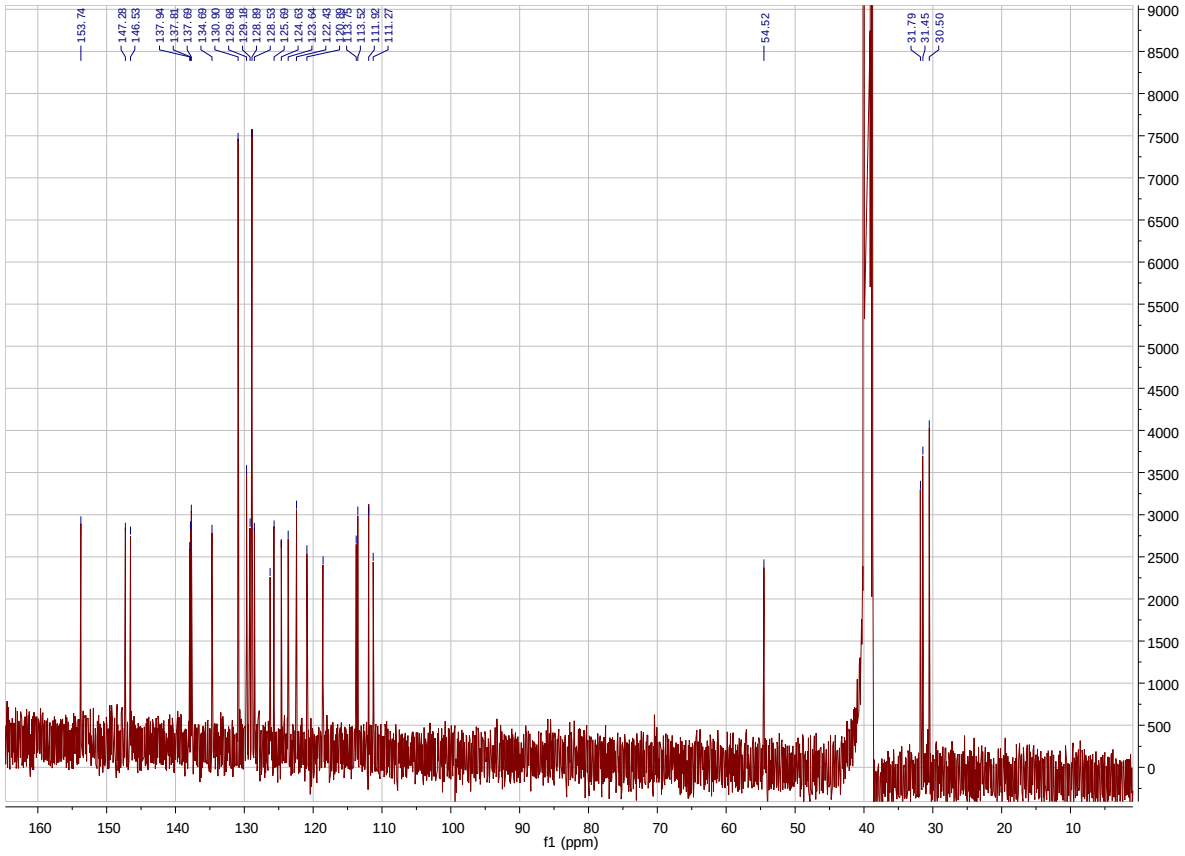
MS (FAB) m/z (%): 481.0 (20) [M^+].
HR-MS (FAB) m/z: calculated for $C_{29}H_{26}N_2Br^+$ [M^+]: 481.1274, found: 481.1276.



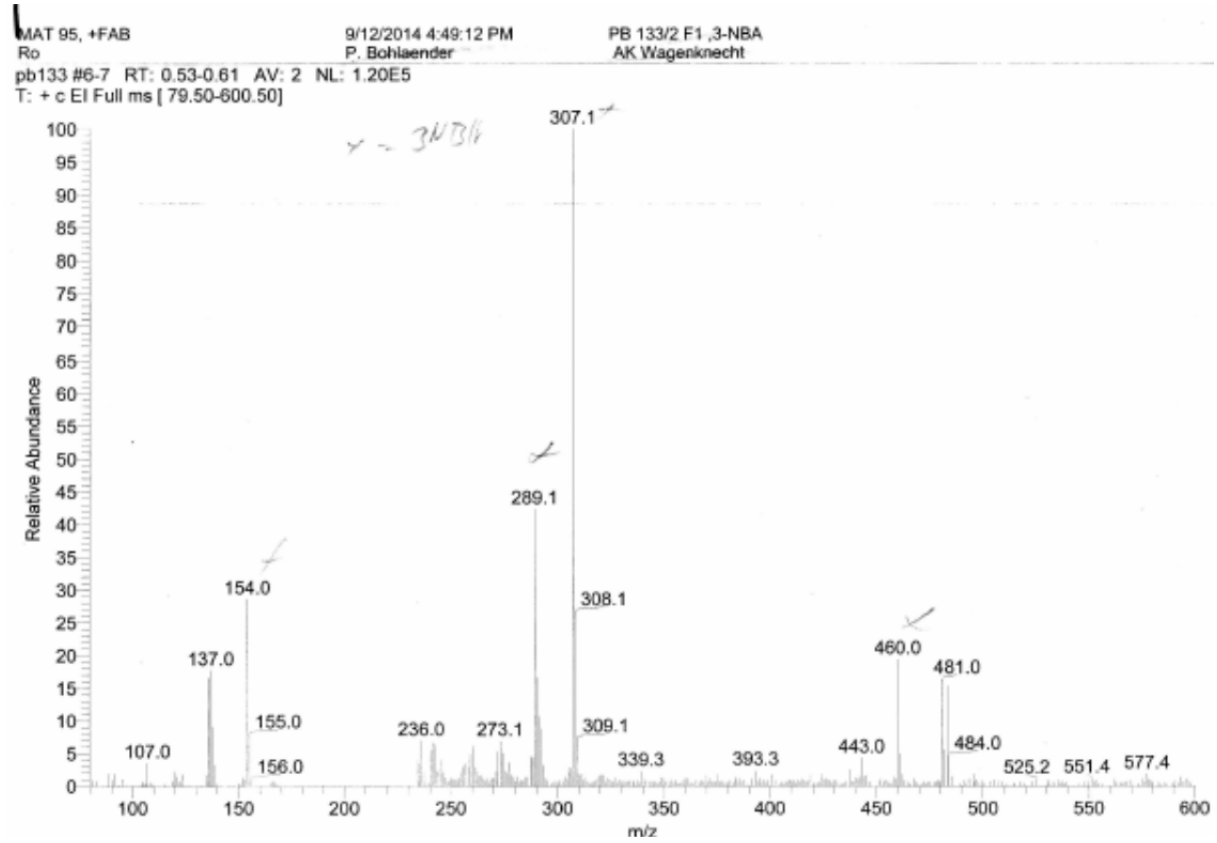
Scheme S22: IR of compound **13**.



Scheme S23: ^{1}H-NMR of compound **13**.



Scheme S24: ^{13}C-NMR of compound **13**.



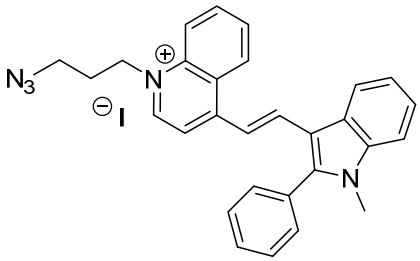
Scheme S25: MS (FAB) of compound **13**.

pb133-c6#10 RT: 0.84
T: + c EI Full ms [79.61-600.61]
m/z= 481.0309-481.1829

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
481.1276	95919.0	100.00	481.1274	0.18	$C_{29}H_{26}N_2{}^{79}Br_1$

Scheme S26: HR-MS (FAB) of compound **13**.

Synthesis of 2



Under argon, a mixture of compound **13** (0.17 g, 0.30 mmol), NaN_3 (0.20 g, 3.00 mmol) and NaI (0.15 g, 1.00 mmol) in 3 mL dimethylformamide was stirred in a headspace vial at room temperature for 19 h. Afterwards the mixture was poured in 200 mL diethylether. The precipitation was collected and washed three times with diethylether. After addition of 2 g NaI the crude product was solubilized in 100 mL water and 100 mL dichloromethane. The aqueous phase was extracted two times with 50 mL dichloromethane. The solvent of the organic phase was removed at 35 °C and reduced pressure. The residue was suspended in 10 mL methanol (use of ultra sonic bath). The precipitation was collected and washed three times with diethylether. Drying under reduced pressure yields a dark-red solid (84 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.45.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3390 (w), 2098 (s), 1580 (m), 1385 (m), 1220 (w).

^{1}H-NMR (400MHz; DMSO-d_6):

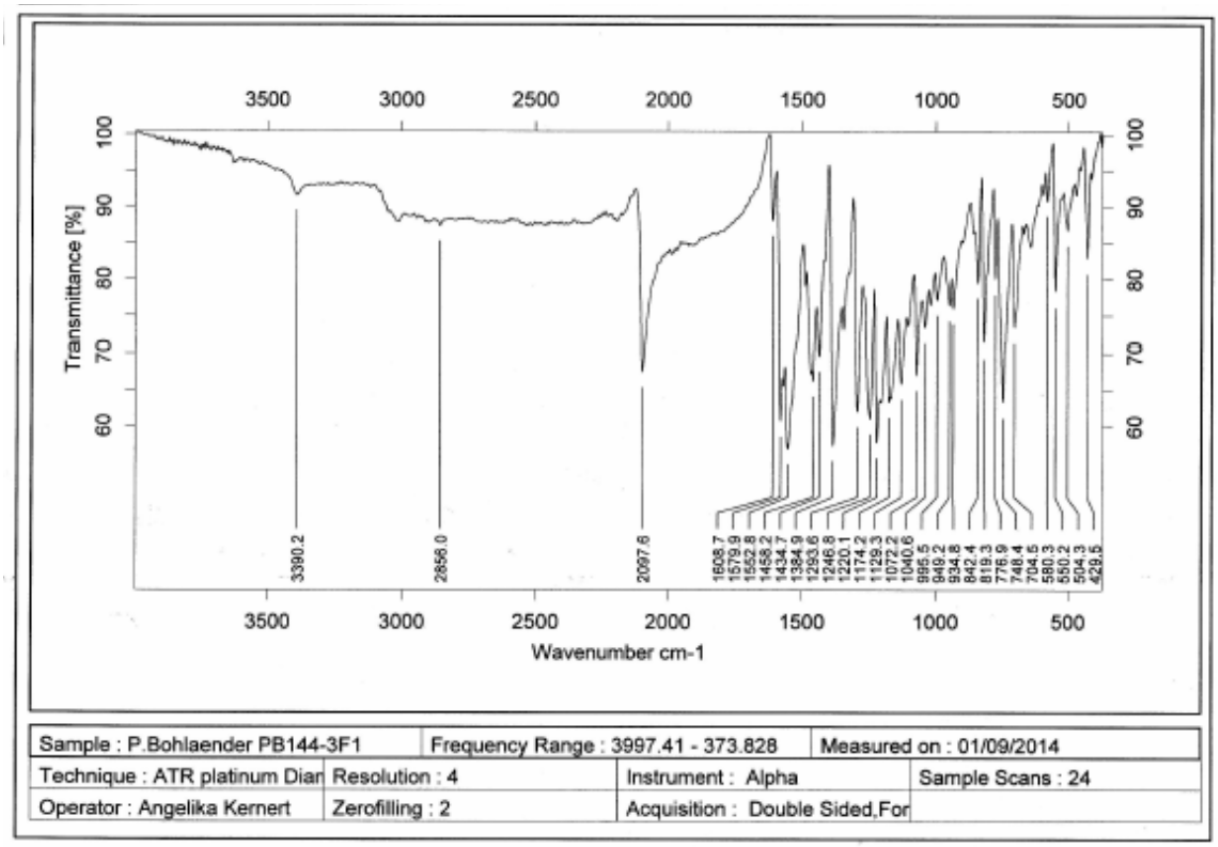
δ (ppm) = 2.16 (p, *J* = 6.7, 2H), 3.54 (t, *J* = 6.6, 2H), 3.72 (s, 3H), 4.90 (t, *J* = 7.3, 2H), 7.43 (p, *J* = 7.1, 2H), 7.62 – 7.74 (m, 6H), 7.84 – 8.01 (m, 4H), 8.19 (t, *J* = 7.9, 1H), 8.41 (t, *J* = 9.0, 2H), 8.69 (d, *J* = 8.5, 1H), 9.01 (d, *J* = 6.7, 1H).

^{13}C-NMR (100 MHz, DMSO-d_6):

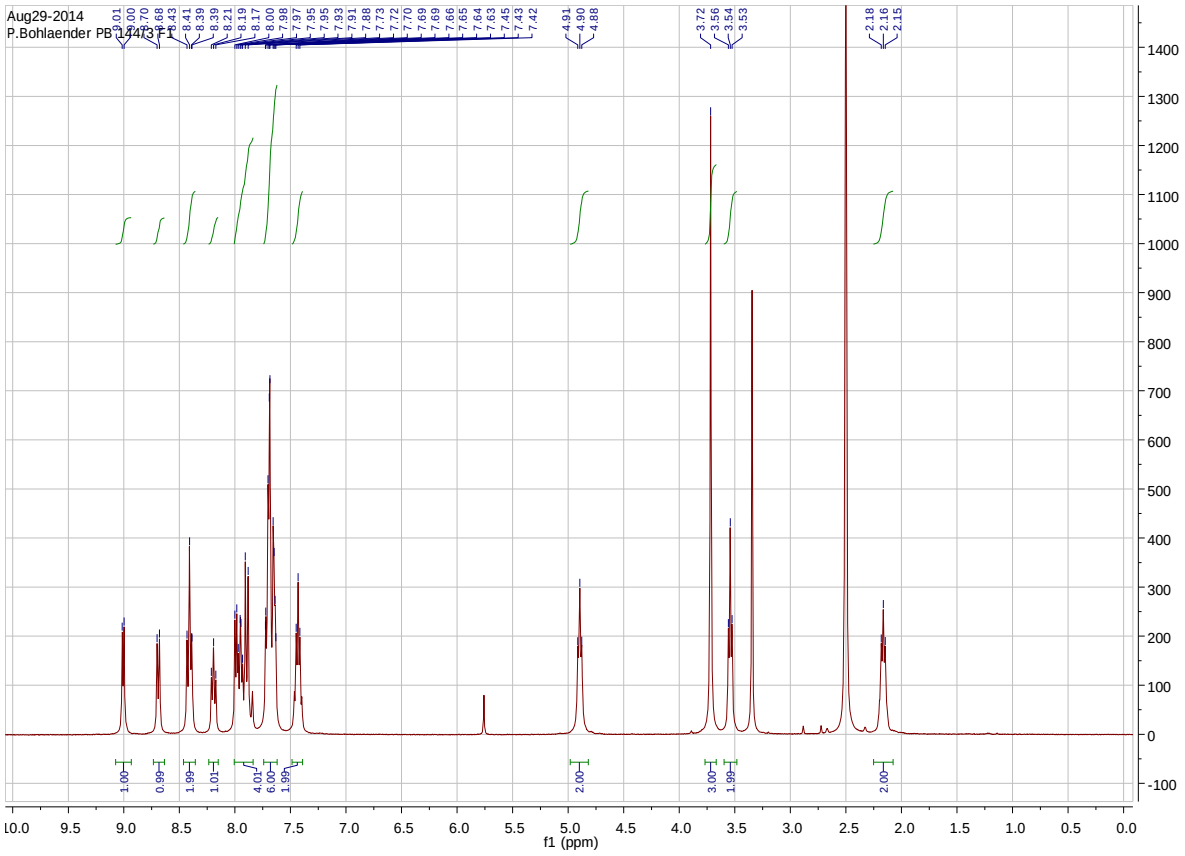
δ (ppm) = 28.2, 31.5, 47.7, 53.5, 111.3, 112.0, 113.6, 113.8, 118.8, 121.0, 122.5, 123.7, 124.7, 125.7, 126.3, 128.6, 129.0, 129.3, 129.8, 131.0, 134.7, 137.7, 137.9, 137.9, 146.5, 147.3, 153.7.

MS (FAB) m/z (%): 444.1 (100) [M^+].

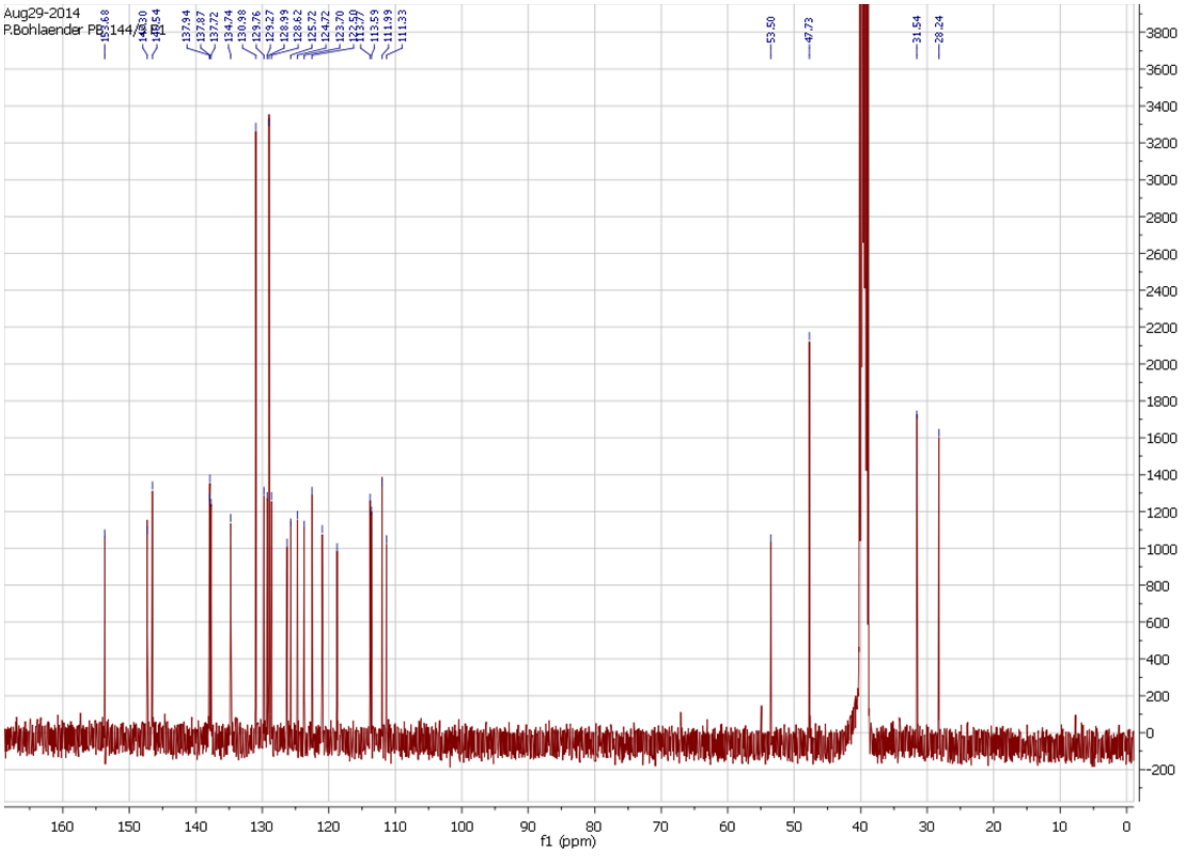
HR-MS (FAB) m/z: calculated for $C_{29}H_{26}N_5^+$ [M^+]: 444.2183, found: 444.2181.



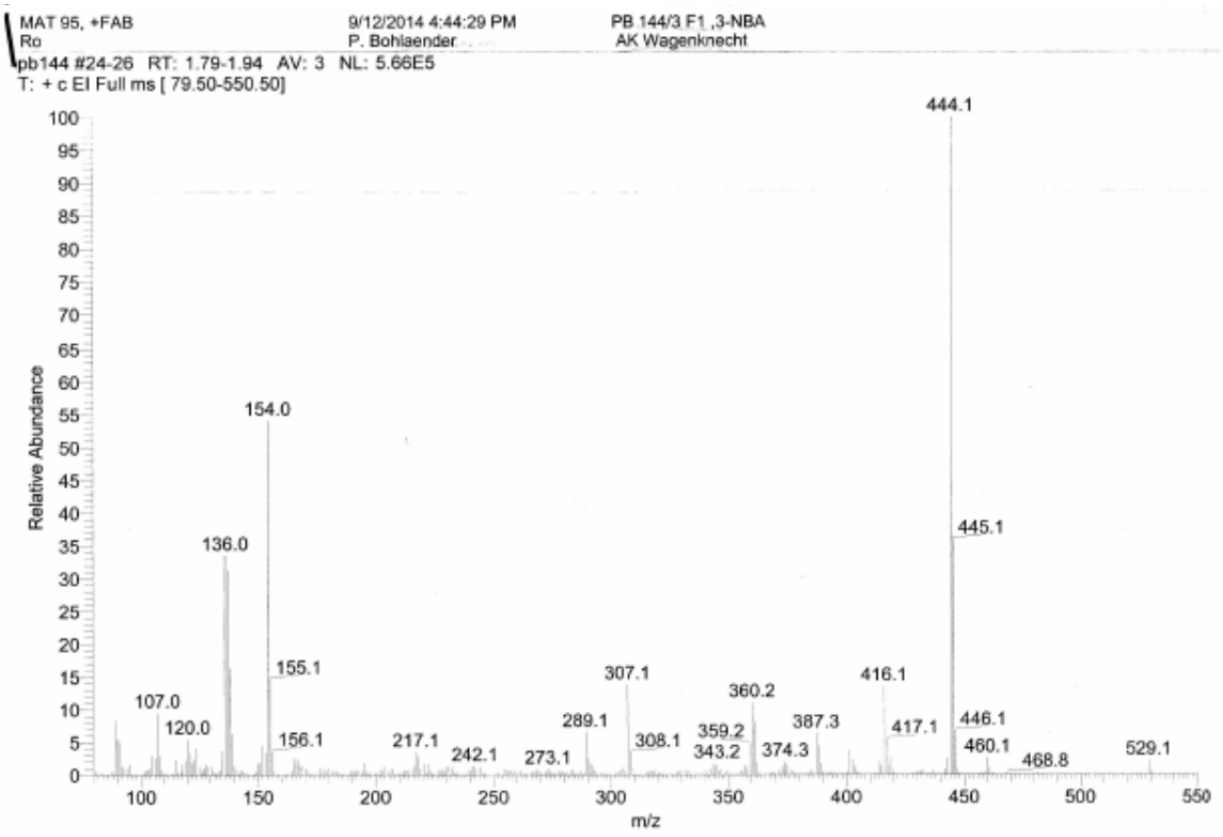
Scheme S27: IR of azide **2**.



Scheme S28: ^{1}H-NMR of azide **2**.



Scheme S29: ^{13}C-NMR of azide **2**.



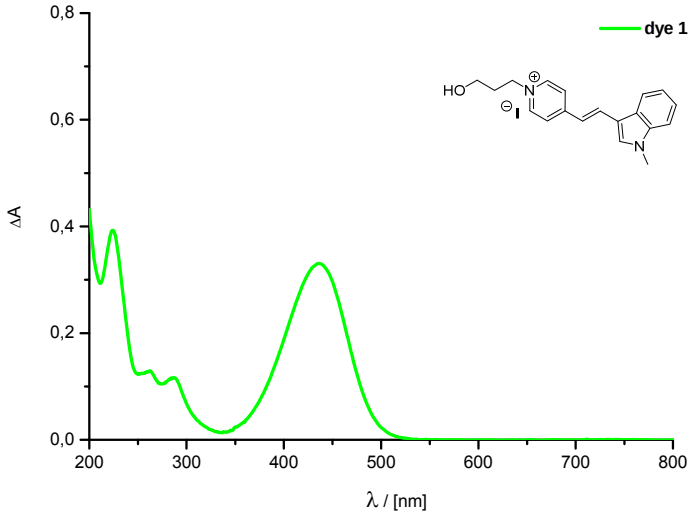
Scheme S30: MS (FAB) of azide **2**.

pb144-c8#7 RT: 0.55
T: + c EI Full ms [79.64-550.64]
m/z= 444.0907-444.3234

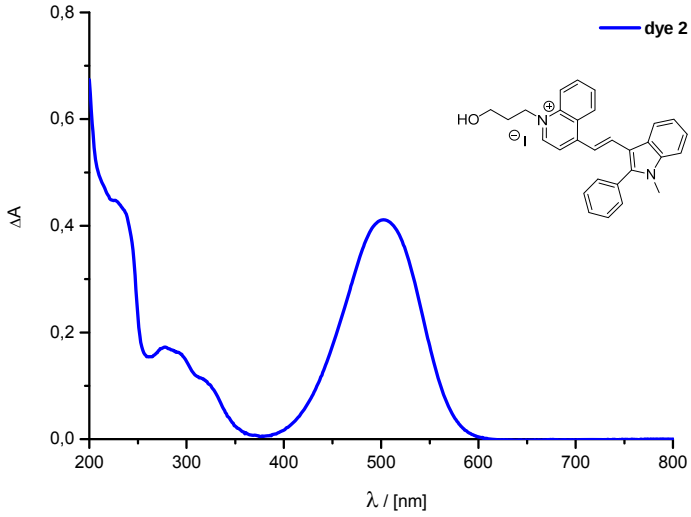
m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
444.2181	391416.0	100.00	444.2183	-0.19	$C_{29}H_{26}N_5$

Scheme S31: HR-MS (FAB) of azide **2**.

4. Absorption spectra of dye 1 and dye 2



Scheme S32: Absorption spectra of dye 1 (c = 11.2 µmol/L in 10% EtOH in water).



Scheme S33: Absorption spectra of dye 2 (c = 13.8 µmol/L in 10% EtOH in water).

5. Photostability

The photostability of dye **1**, dye **2**, **TR** and **TO** was observed by the loss of fluorescence intensity in the presence of random sequence double stranded unmodified DNA (10 µM dye*, 2.5 µM **DNA23**, 10 mM NaP_i (pH = 7), 250 mM NaCl and 5 % ethanol). The solution was irradiated with a 75 W Xe-arc lamp equipped with a 305 nm cutoff filter to avoid excitation of the DNA components. The fluorescence intensity was recorded at 20 °C after mixing the irradiated sample solution.

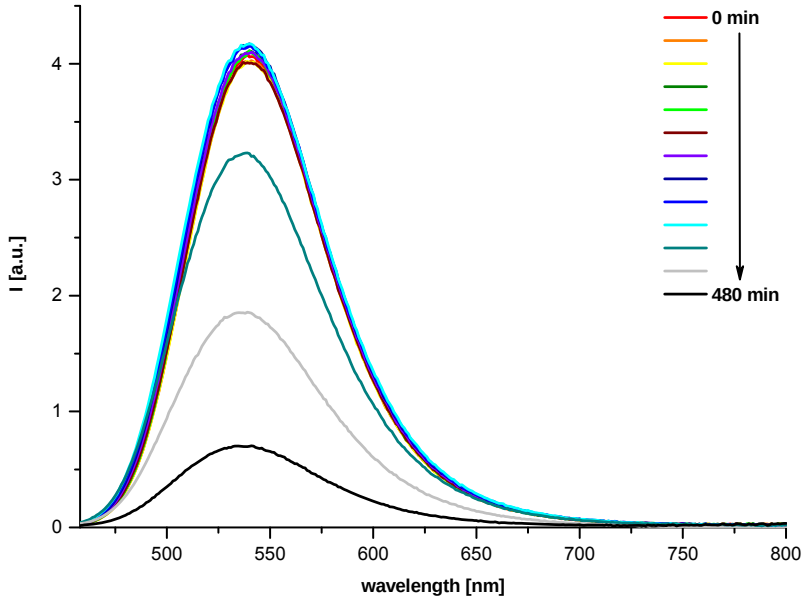
*The preparation of a 50 µM dye solution: The required amount of dye was weighed in 50 mL volumetric flasks, respectively. After adding 5 mL pure ethanol the mixture was treated 10 to 15 minutes in an ultrasonic bath to ensure that the dye was quantitatively solubilized. In the next step the solution was diluted with water to 50 mL. Afterwards the solution was diluted to get the required concentration of the dye and ethanol.

5´- TCA-GTG-ATC-TAG-ACT-GC - 3´

3´- AGT-CAC-TAG-ATC-TGA-CG - 5

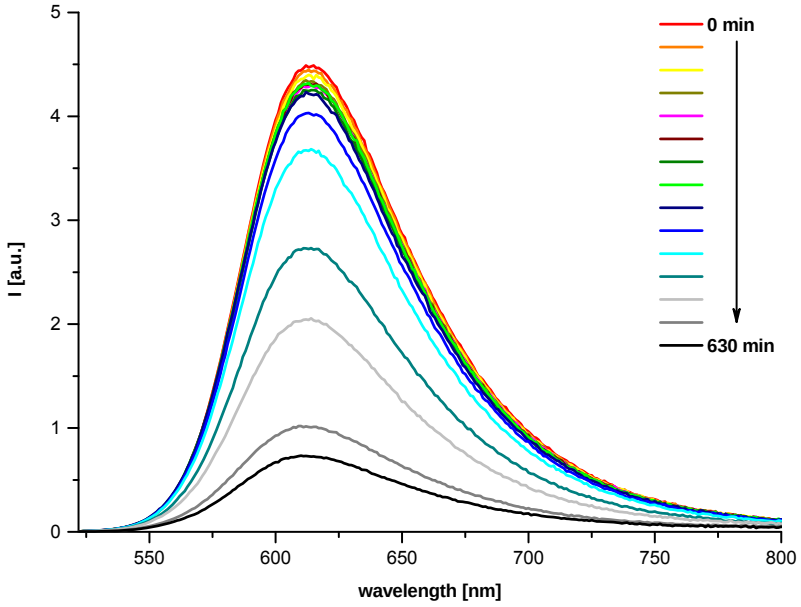
Scheme S34: Sequence of **DNA23**.

5.1 Photostability of dye 1:



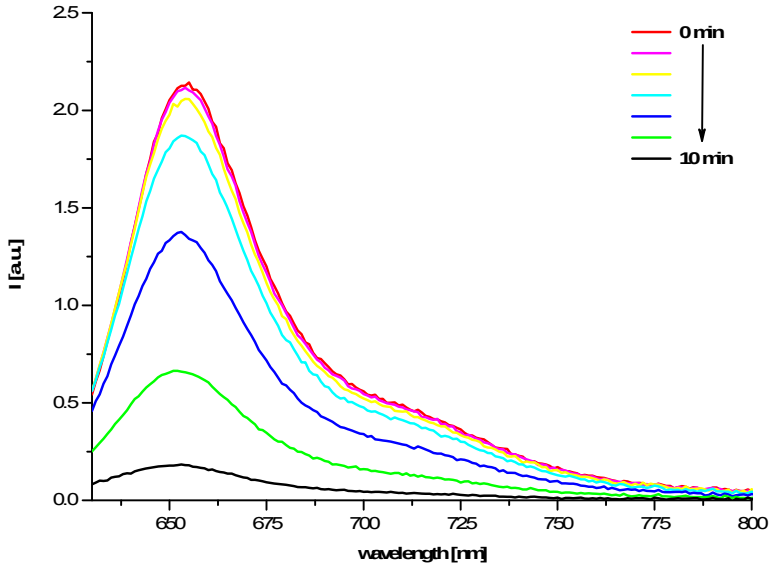
Scheme S35: Fluorescence spectra of the photodegradation of dye **1**, $\lambda_{exc.}$ = 436 nm, $\lambda_{em.,\,max.}$ = 539 nm, $t_{1/2}$ = 293 min.

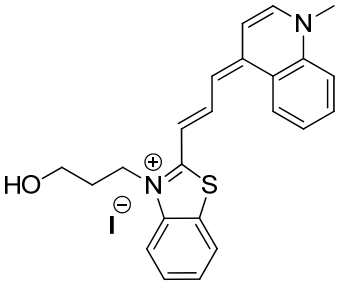
5.2 Photostability of dye 2:



Scheme S36: Fluorescence spectra of the photodegradation of dye **2**,
$\lambda_{exc.}$ = 507 nm, $\lambda_{em.,\,max.}$ = 615 nm, $t_{1/2}$ = 317 min.

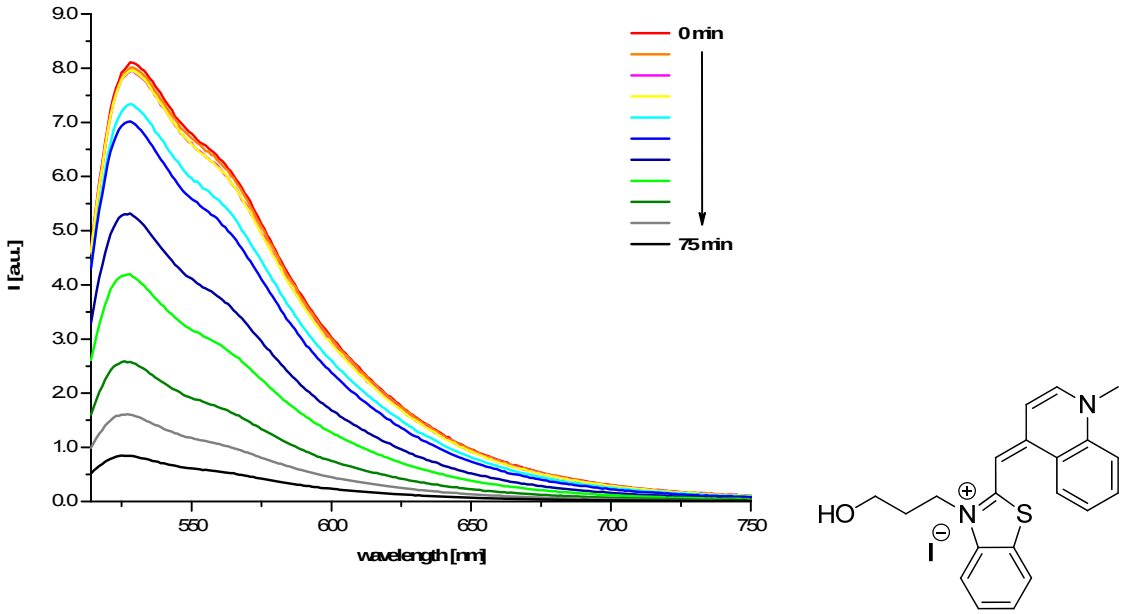
5.3 Photostability of TR:





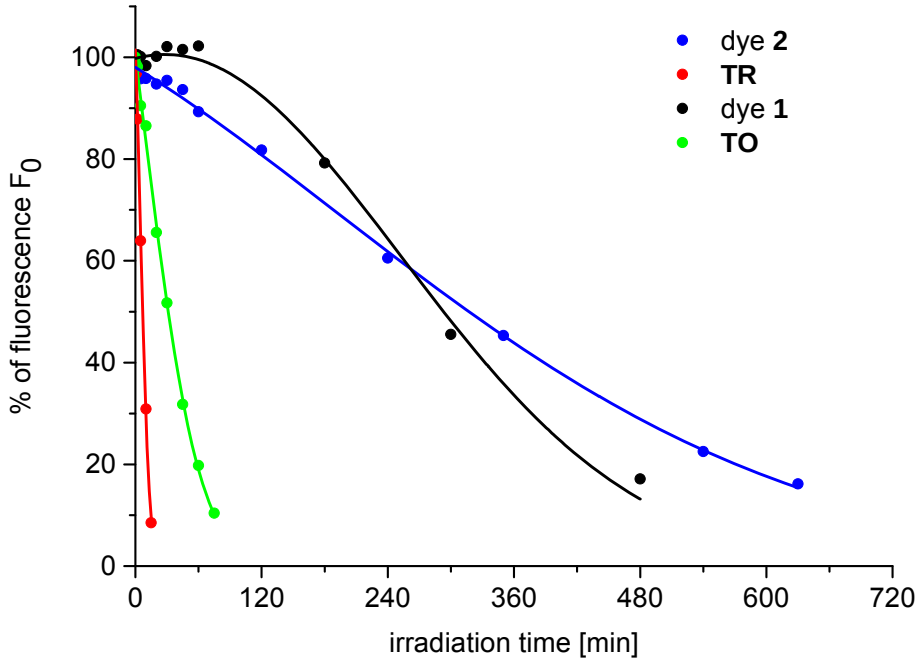
Scheme S37: Fluorescence spectra of the photodegradation of **TR**,
$\lambda_{exc.}$ = 620 nm, $\lambda_{em.,\,max.}$ = 654 nm, $t_{1/2}$ = 7 min (left); structure of **TR** (right).[2]

5.4 Photostability of TO:



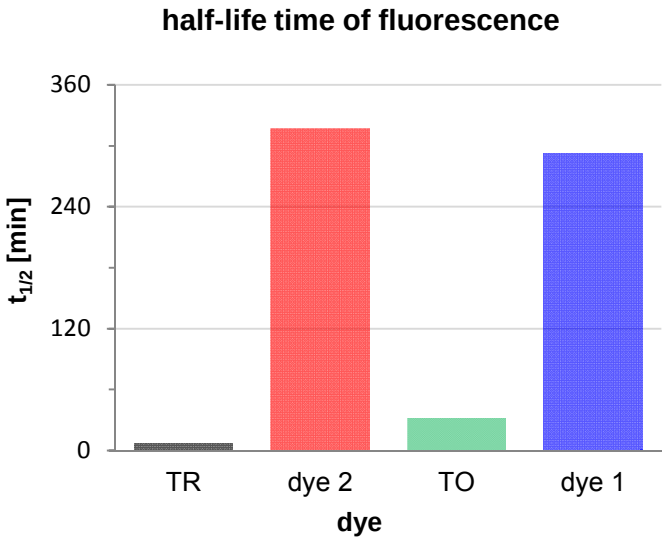
Scheme S38: Fluorescence spectra of the photodegradation of **TO**, $\lambda_{exc.}$ = 494 nm, $\lambda_{em., max.}$ = 528 nm, $t_{1/2}$ = 32 min (left); structure of **TO** (right).[2]

5.5 Comparison of the photostabilities and half-life times ($t_{1/2}$):



Scheme S39: Photostability of dye **1**, dye **2**, **TR** and **TO** (% of fluorescence intensity F_0).

dye	half-life time $t_{1/2}$ [min]
TR	7
dye **2**	317
TO	32
dye **1**	293



Scheme S40: Half-life time $t_{1/2}$ [min] of dye **1**, dye **2**, **TR** and **TO**.

6. Preparation and purification of DNA1-DNA22

All oligonucleotides were synthesized on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry. Reagents and CPG (1 µmol) were purchased from Proligo. The commercially available 2'-*O*-propargyl-uridine (cU) was purchased from ChemGenes. The acyclic linker (cL) was synthesized according to literature procedures.[??] The coupling time for cL was extended to 10 min. After preparation, the trityl-off oligonucleotides were cleaved from the resin and deprotected with conc. NH_4OH at 45 °C for 16 h.

6.1 Click reaction of dyes 1 and 2 with cU- and cL-modified oligonucleotides

To the lyophilized alkyne-modified DNA sample were added 50 µL water, 25 µL of a sodium ascorbate solution (0.4 M in water), 34 µL tris-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (0.1 M in DMSO/t-BuOH 3:1), 114 µL of the azide (0.01 M in DMSO/t-BuOH 3:1) and finally 17 µL of a tetrakis(acetonitrile) copper(I)hexafluorophosphate solution (0.1 M in DMSO/t-BuOH 3:1). The reaction mixture was kept at 60 °C for 1.5 h. After cooling to room temperature, the DNA was precipitated by adding 150 µL Na_2EDTA (0.05 M in water), 450 µL sodium acetate (0.3 M in water) and 10 mL ethanol (100%) and stored at -32 °C for 16 h. After centrifugation the supernatant was removed and the residue washed two times with 2 mL cold ethanol (80%). The dried DNA pellet was then further purified via HPLC.

6.2 HPLC-purification of DNA1-DNA22

The labelled oligonucleotides were purified via HPLC Reversed Phase Supelcosil™ LC-C18 column (250 x 10 mm, 5 µm) on a Shimadzu HPLC sytem (autosampler, SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A) using the following conditions:

eluent A:	NH_4OAc buffer (0.05 M in water, pH 6.5)
eluent B:	acetonitrile
flow rate:	2.5 mL/min

For gradients see Table S39. UV/Vis detection at 260 nm, 459 nm for oligonucleotides modified with dye **1**, 542 nm for oligonucleotides modified with dye **2**.

time [min]	eluent B [%]
0	0
45	15[a]/17[b]
65	15[a]/17[b]
66	90
75	90
76	0
85	0

Table S41: HPLC-gradients for semi-preparative purification of oligonucleotides modified with [a] dye **1** and [b] dye **2**.

7. HPLC-analytic of purified DNA1 and DNA2

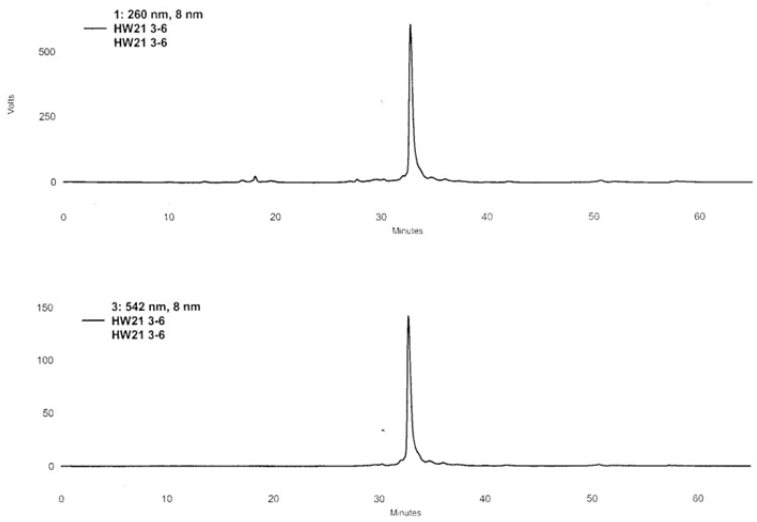
Analytical HPLC of the purified DNA samples were performed with Reversed phase Supelcosil^{TM} LC-C18 column (250 x 4.5 mm, 5 µm) on a Shimadzu HPLC sytem (autosampler, SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A) using the following conditions:

eluent A: NH_4OAc buffer (0.05 M in water, pH 6.5)
eluent B: acetonitrile
flow rate: 1.0 mL/min

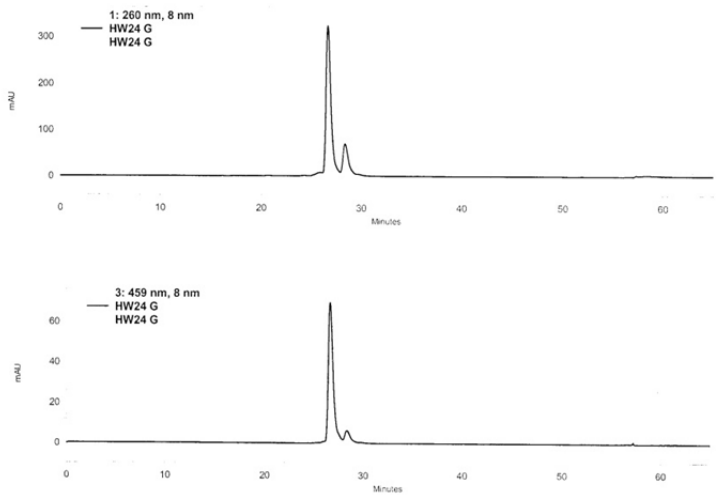
For gradients see Table S40. UV/Vis detection at 260 nm, 459 nm for oligonucleotides modified with dye **1**, 542 nm for oligonucleotides modified with dye **2**.

time [min]	eluent B [%]
0	0
45	20[a]/25[b]
50	20[a]/25[b]
51	90
60	90
61	0
65	0

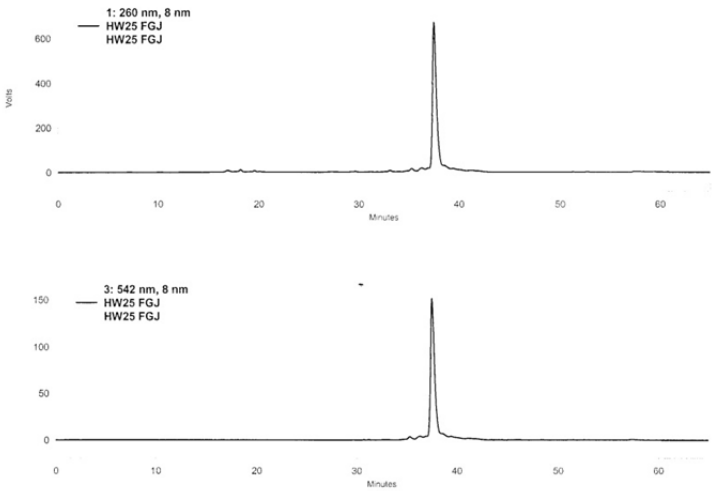
Table S42: HPLC-gradients for analytical determination of purified oligonucleotides modified with [a] dye **1** and [b] dye **2**.



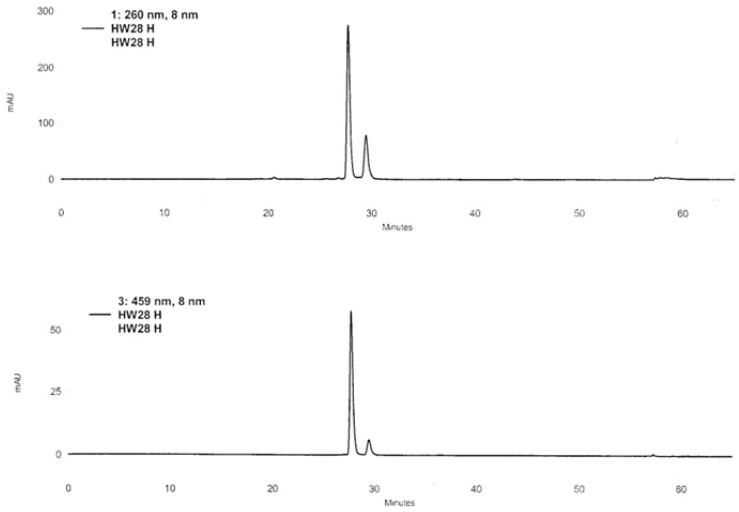
Scheme S43: HPLC-chromatogram of purified **DNA1**.



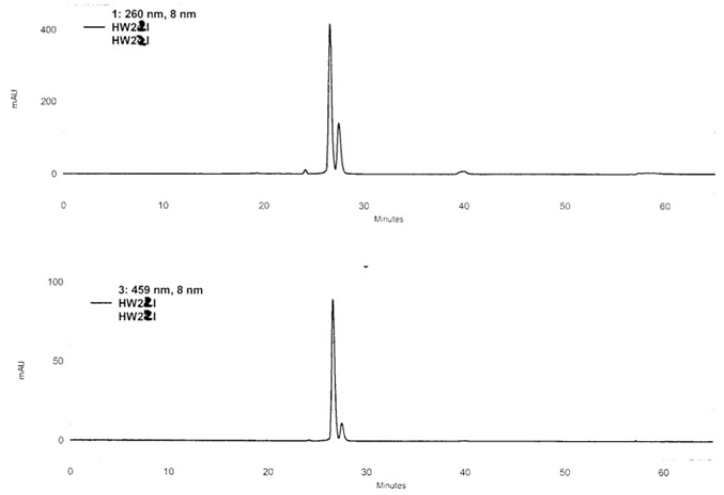
Scheme S44: HPLC-chromatogram of purified **DNA2**.



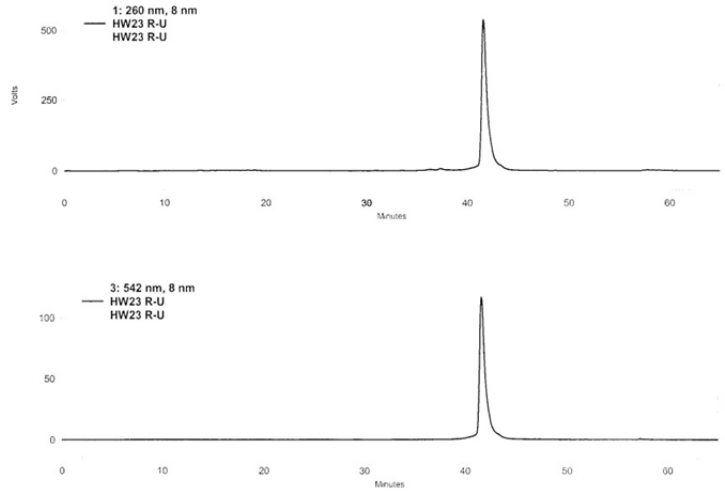
Scheme S45: HPLC-chromatogram of purified **DNA3**.



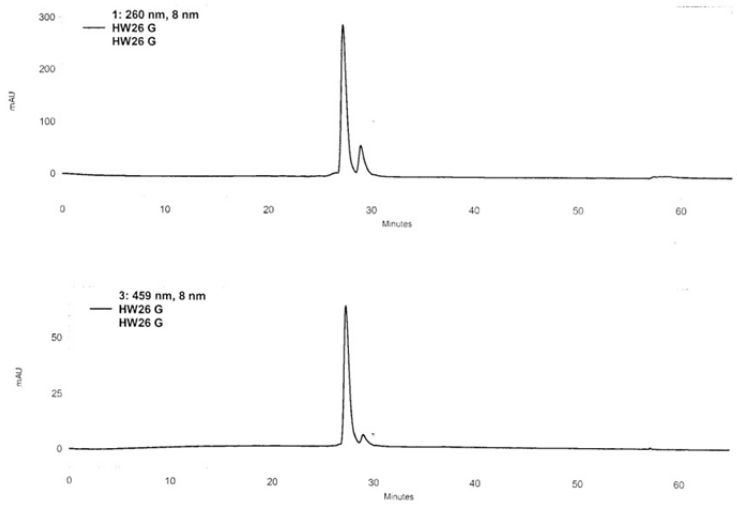
Scheme S46: HPLC-chromatogram of purified **DNA4**.



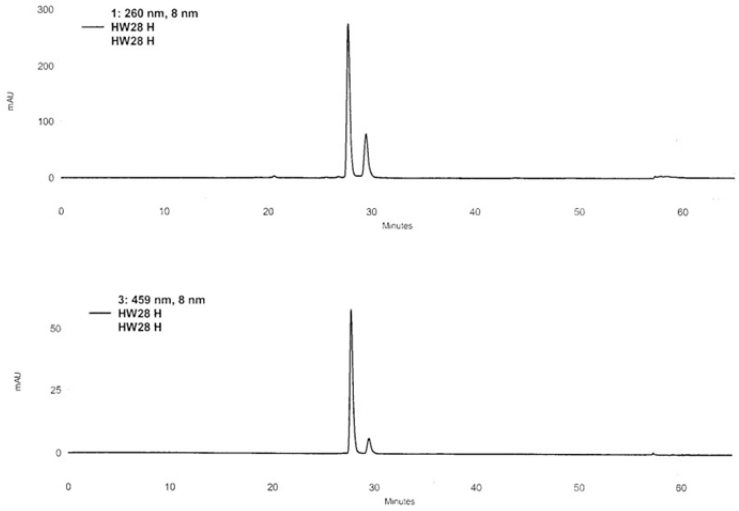
Scheme S47: HPLC-chromatogram of purified **DNA5**.



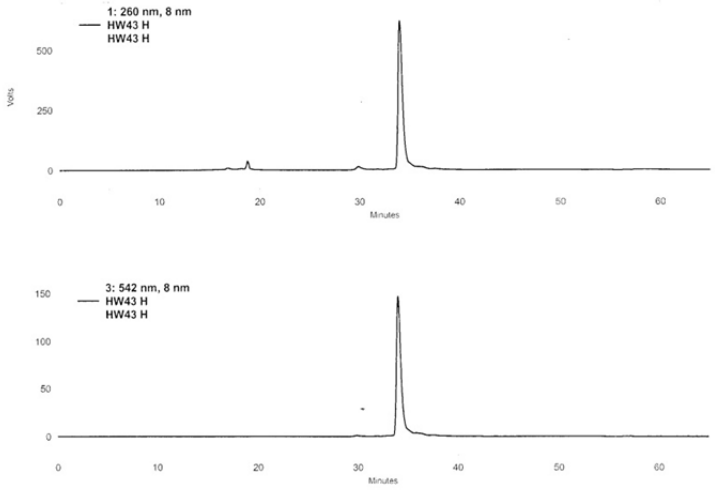
Scheme S48: HPLC-chromatogram of purified **DNA6**.



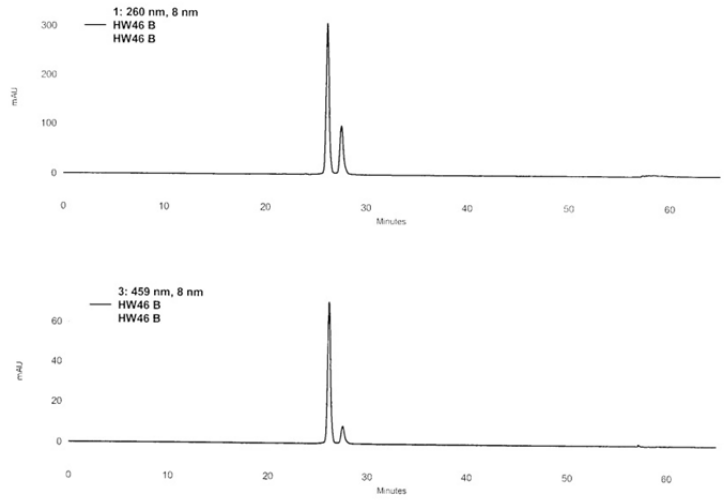
Scheme S49: HPLC-chromatogram of purified **DNA7**.



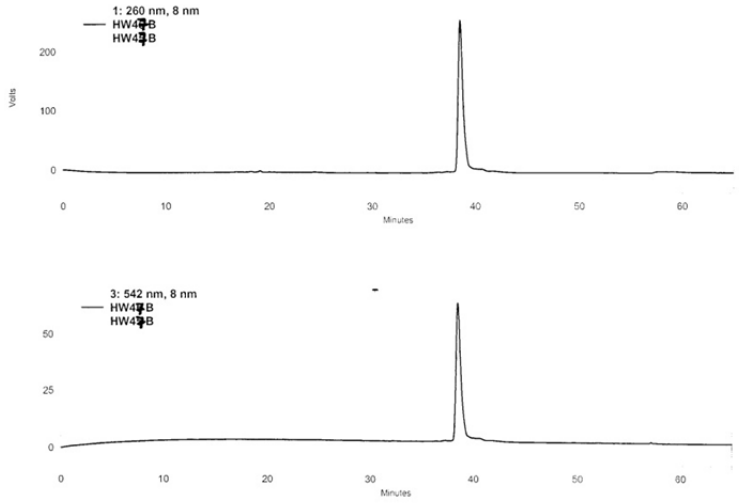
Scheme S50: HPLC-chromatogram of purified **DNA8**.



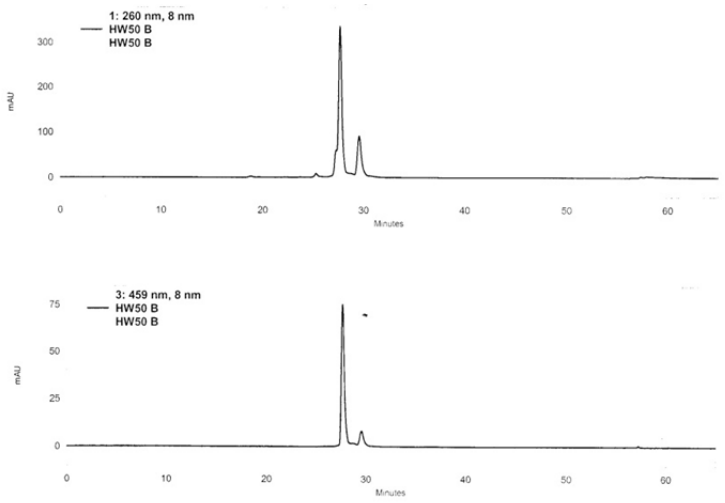
Scheme S51: HPLC-chromatogram of purified **DNA9**.



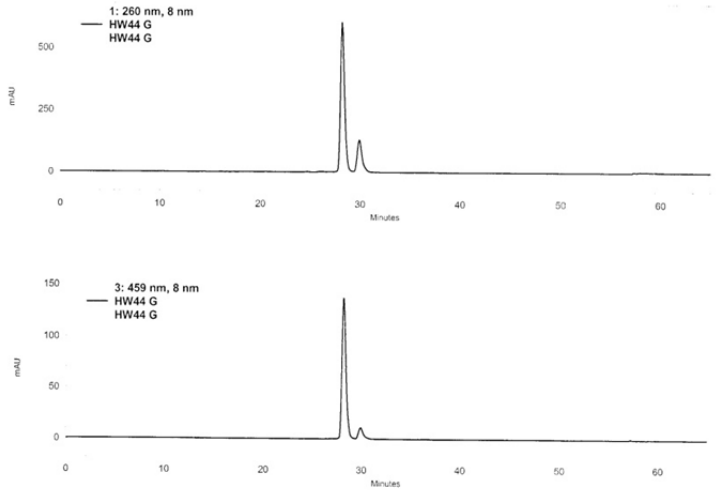
Scheme S52: HPLC-chromatogram of purified **DNA10**.



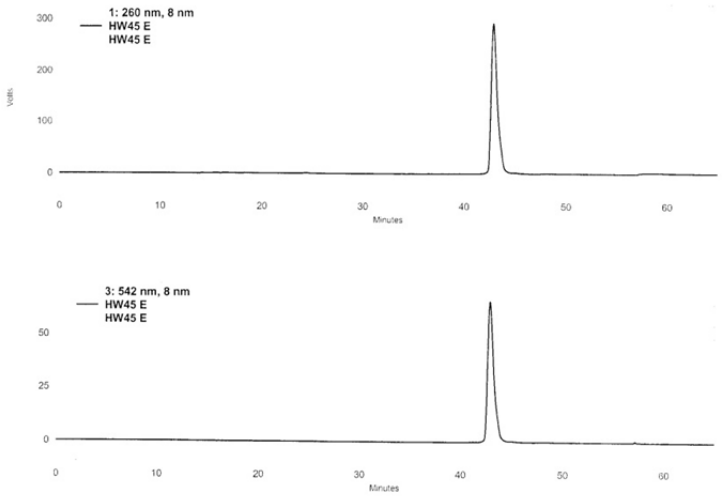
Scheme S53: HPLC-chromatogram of purified **DNA11**.



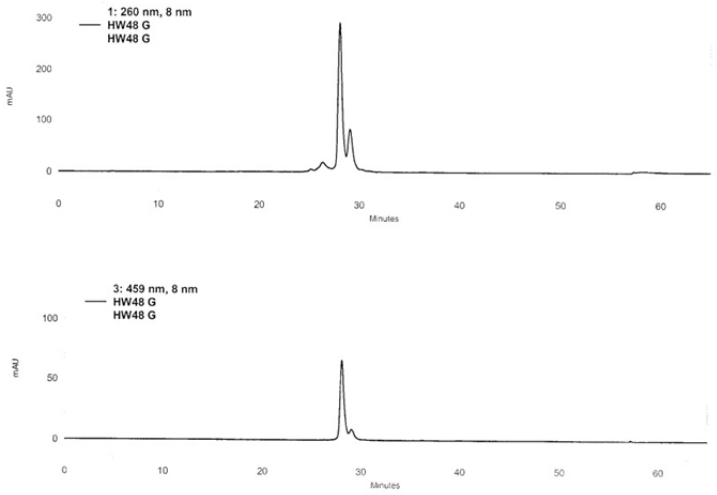
Scheme S54: HPLC-chromatogram of purified **DNA12**.



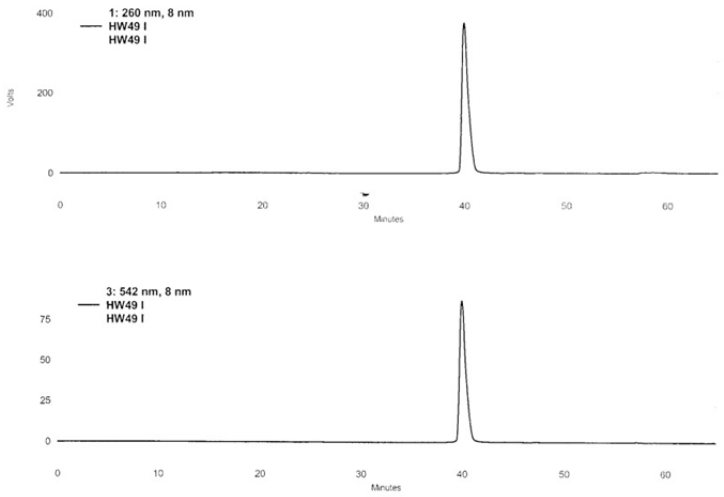
Scheme S55: HPLC-chromatogram of purified **DNA13**.



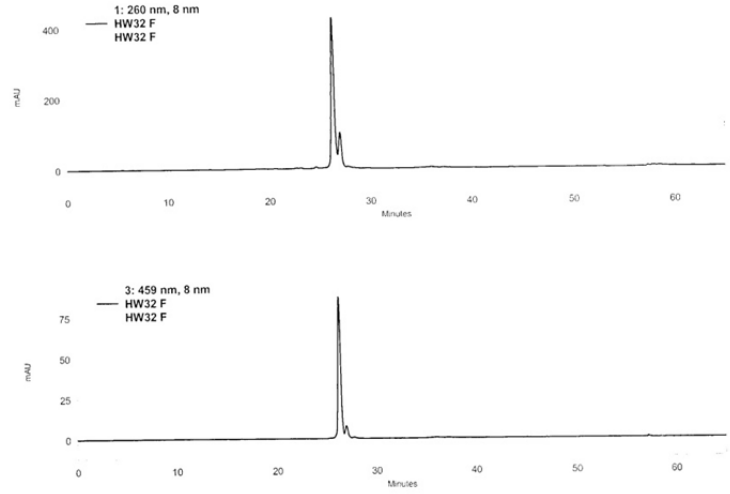
Scheme S56: HPLC-chromatogram of purified **DNA14**.



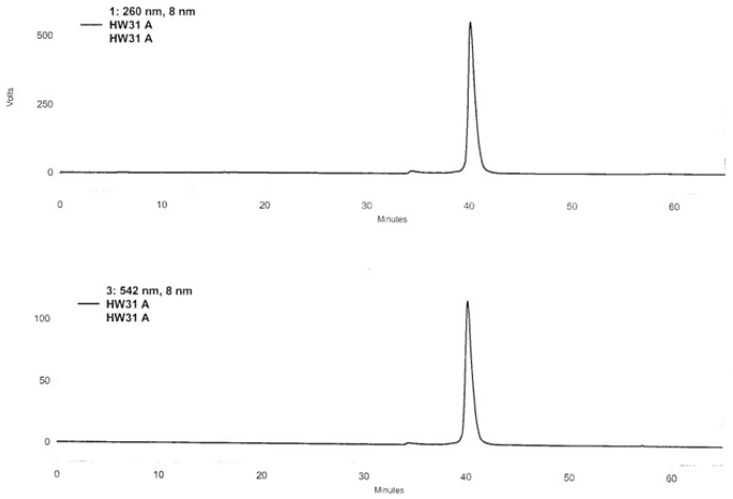
Scheme S57: HPLC-chromatogram of purified **DNA15**.



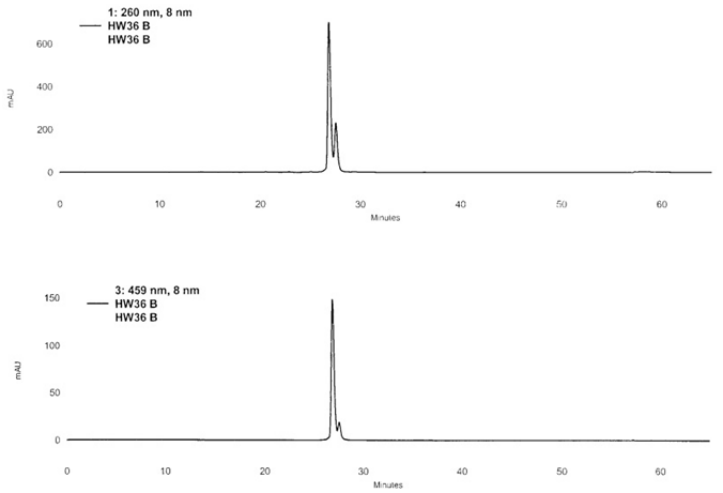
Scheme S58: HPLC-chromatogram of purified **DNA16**.



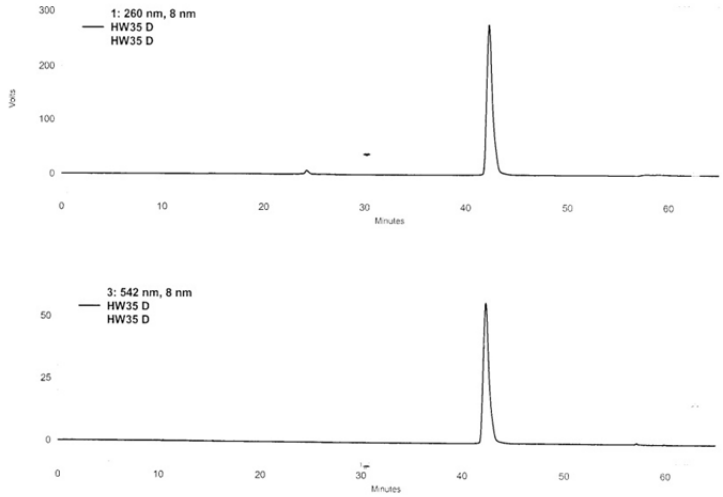
Scheme S59: HPLC-chromatogram of purified **DNA17**.



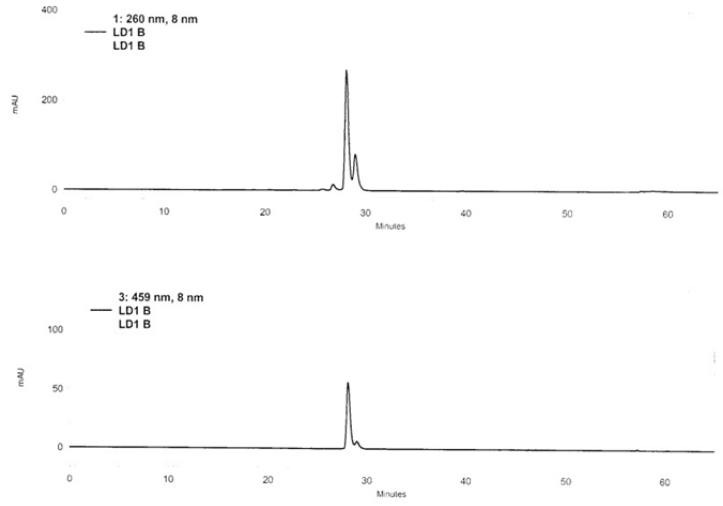
Scheme S60: HPLC-chromatogram of purified **DNA18**.



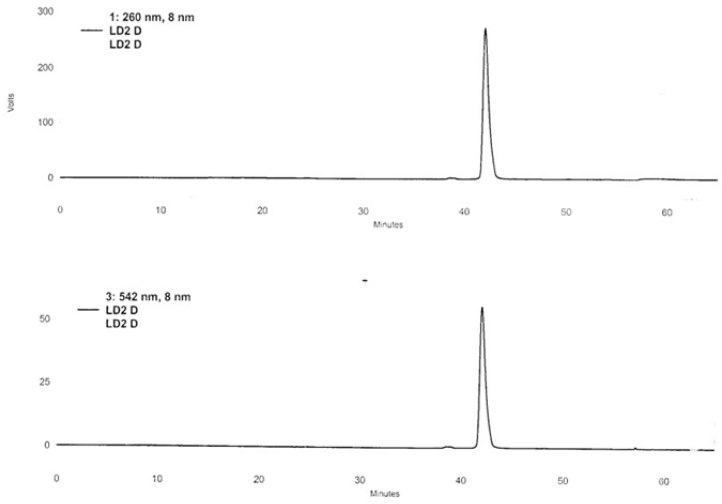
Scheme S61: HPLC-chromatogram of purified **DNA19**.



Scheme S62: HPLC-chromatogram of purified **DNA20**.

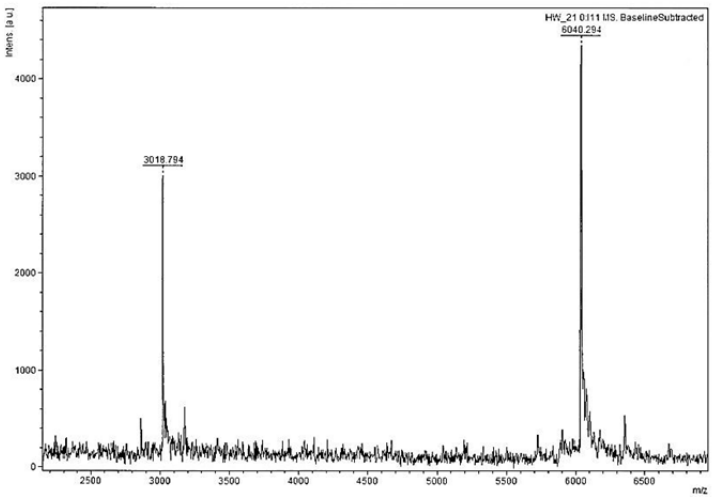


Scheme S63: HPLC-chromatogram of purified **DNA21**.

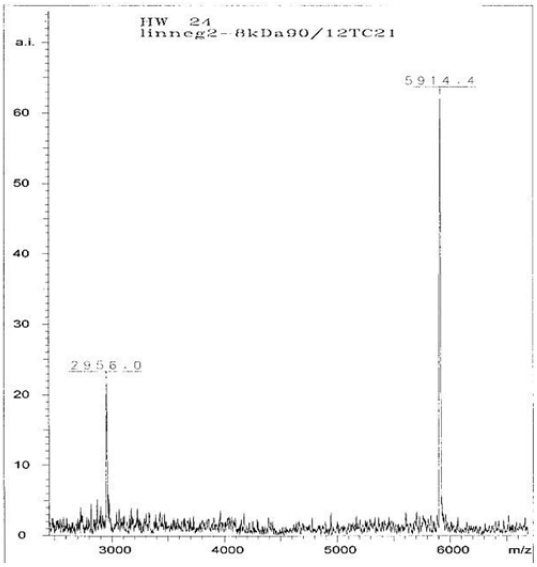


Scheme S64: HPLC-chromatogram of purified **DNA22**.

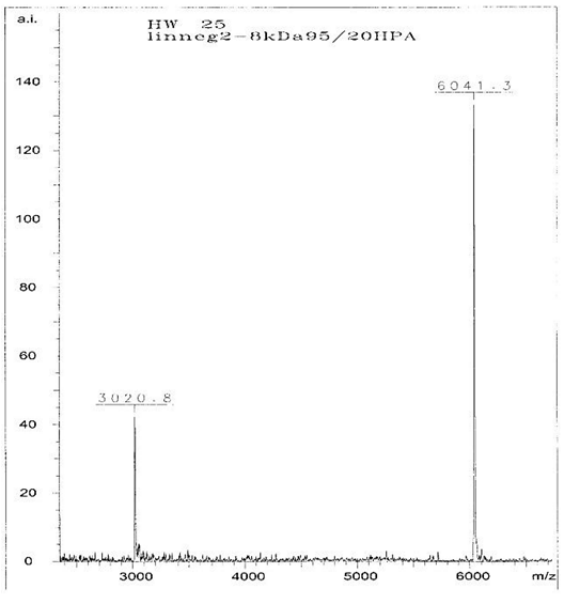
8. MALDI-spectra of purified DNA1-DNA22



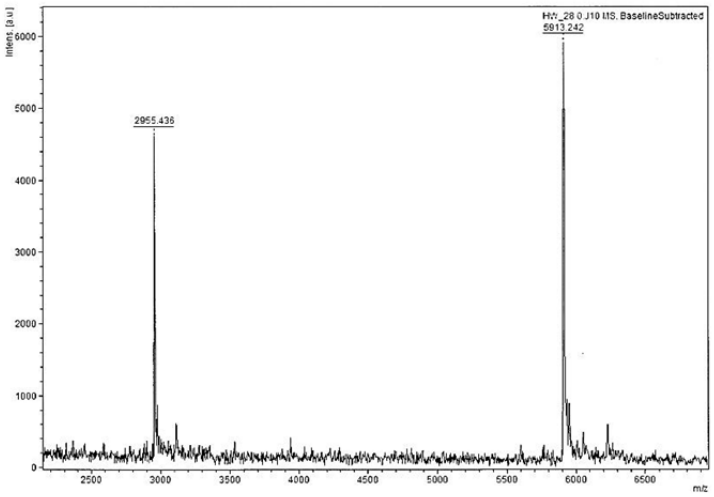
Scheme S65: MALDI-spectra of purified **DNA1**, calculated: 6036.2 Da, found: 6040.3 Da.



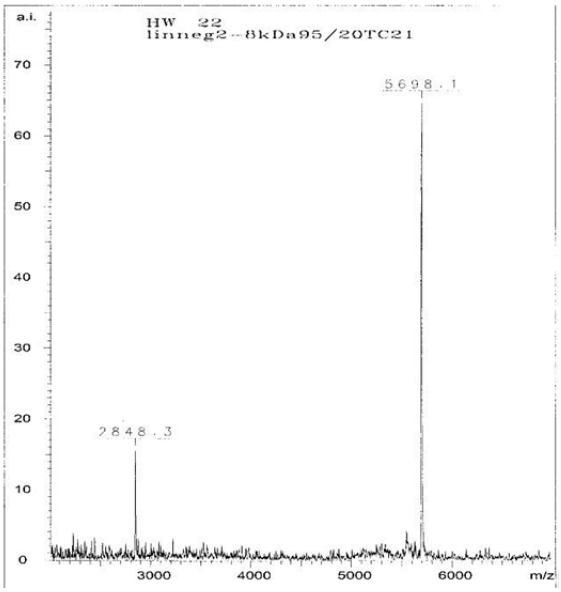
Scheme S66: MALDI-spectra of purified **DNA2**, calculated: 5910.2 Da, found: 5914.4 Da.



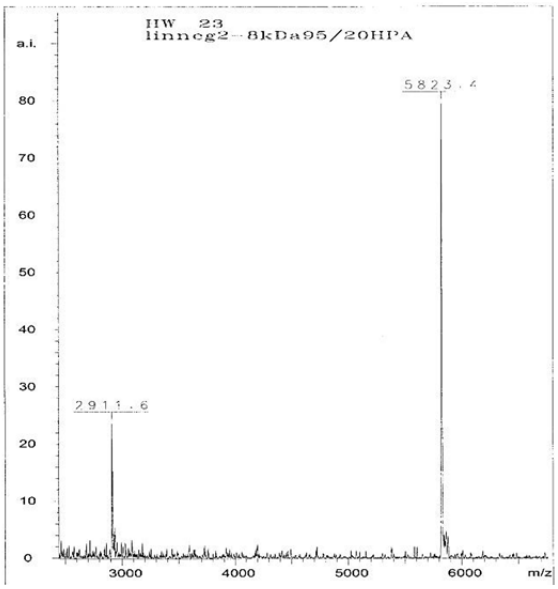
Scheme S67: MALDI-spectra of purified **DNA3**, calculated: 6036.2 Da, found: 6041.3 Da.



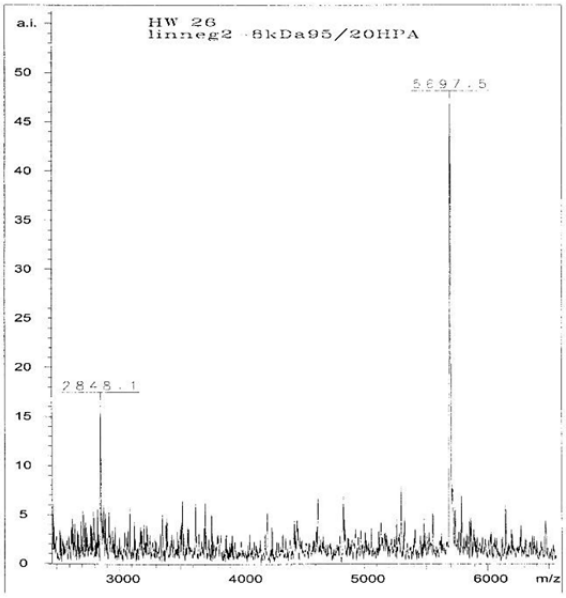
Scheme S68: MALDI-spectra of purified **DNA4**, calculated: 5910.2 Da, found: 5913.2 Da.



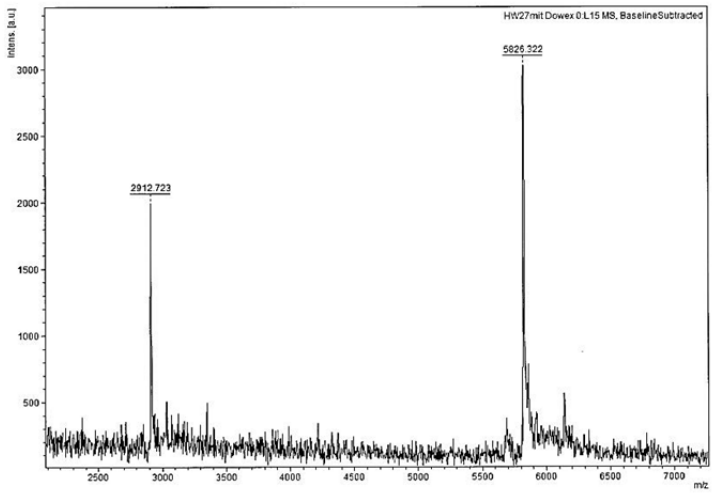
Scheme S69: MALDI-spectra of purified **DNA5**, calculated: 5695.1 Da, found: 5698.1 Da.



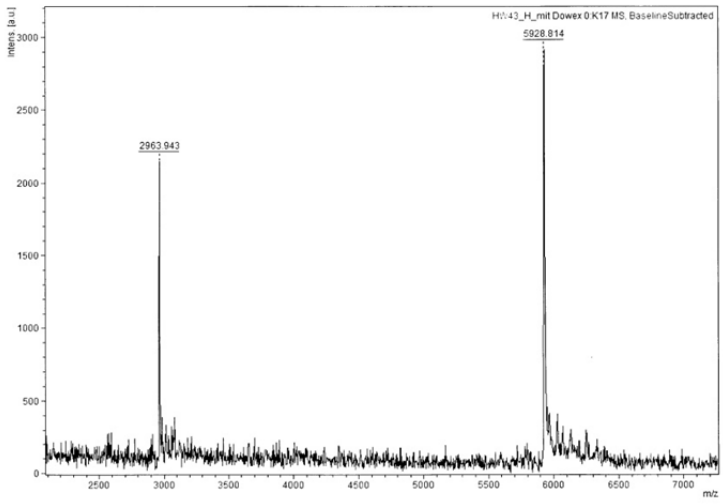
Scheme S70: MALDI-spectra of purified **DNA6**, calculated: 5821.2 Da, found: 5823.4 Da.



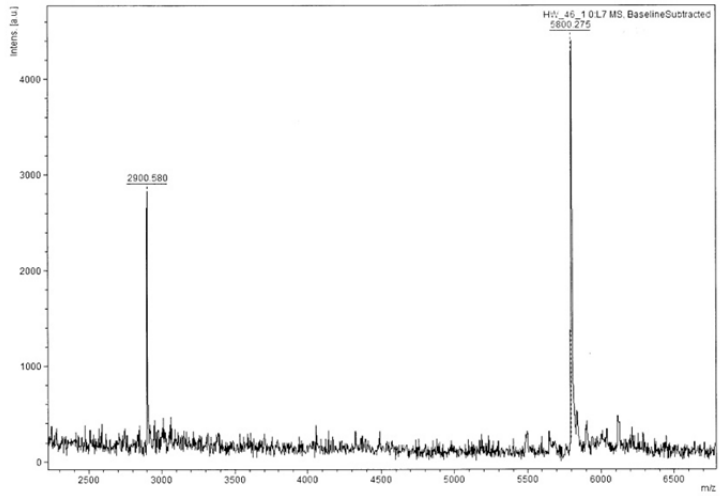
Scheme S71: MALDI-spectra of purified **DNA7**, calculated: 5695.1 Da, found: 5697.5 Da.



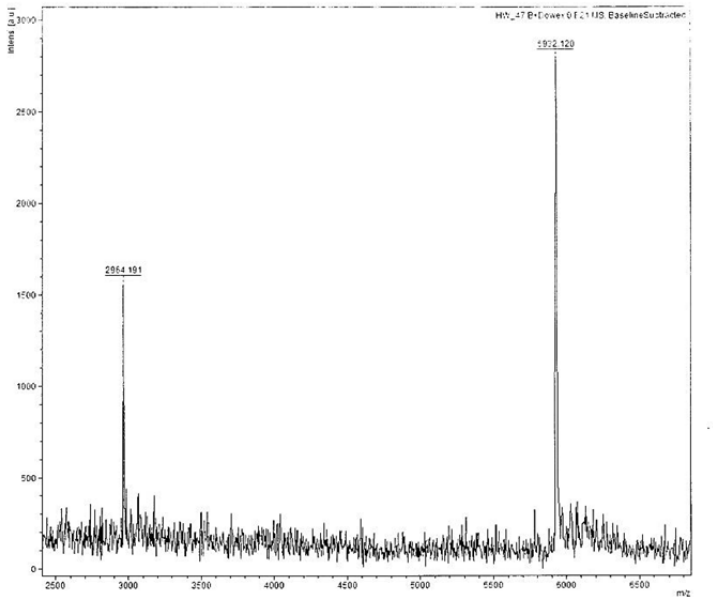
Scheme S72: MALDI-spectra of purified **DNA8** calculated: 5821.2 Da, found: 5826.3 Da.



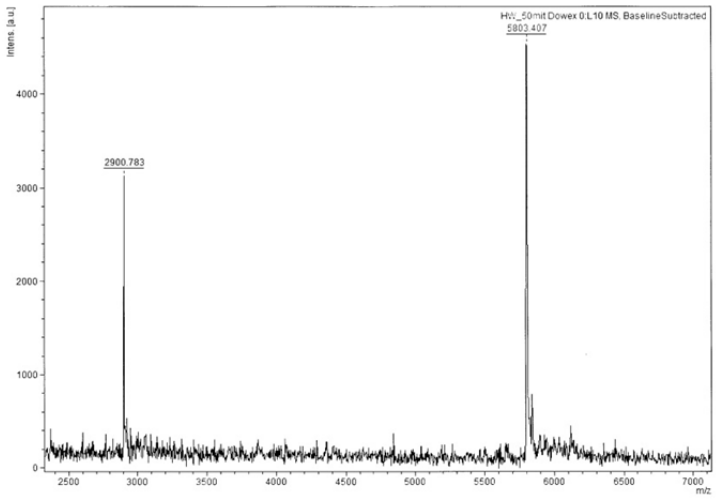
Scheme S73: MALDI-spectra of purified **DNA9**, calculated: 5927.6 Da, found: 5928.8 Da.



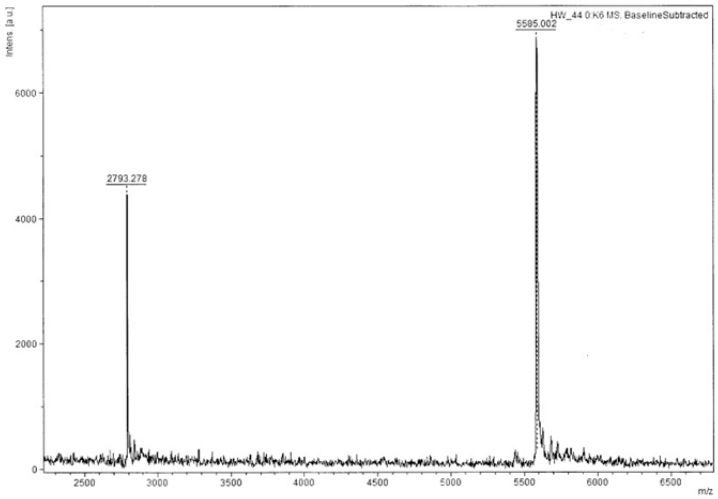
Scheme S74: MALDI-spectra of purified **DNA10**, calculated: 5801.4 Da, found: 5800.3 Da.



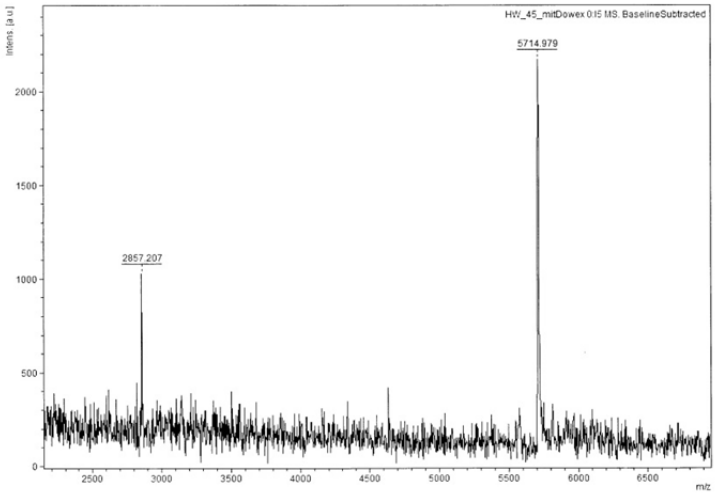
Scheme S75: MALDI-spectra of purified **DNA11**, calculated: 5927.6 Da, found: 5932.1 Da.



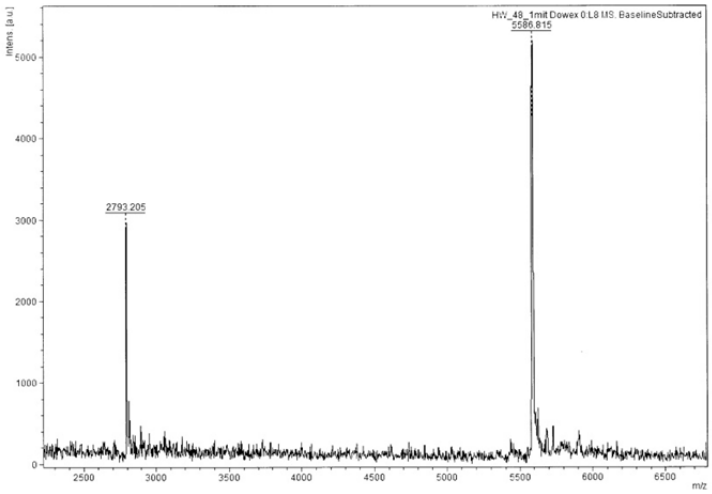
Scheme S76: MALDI-spectra of purified **DNA12**, calculated: 5801.4 Da, found: 5803.4 Da.



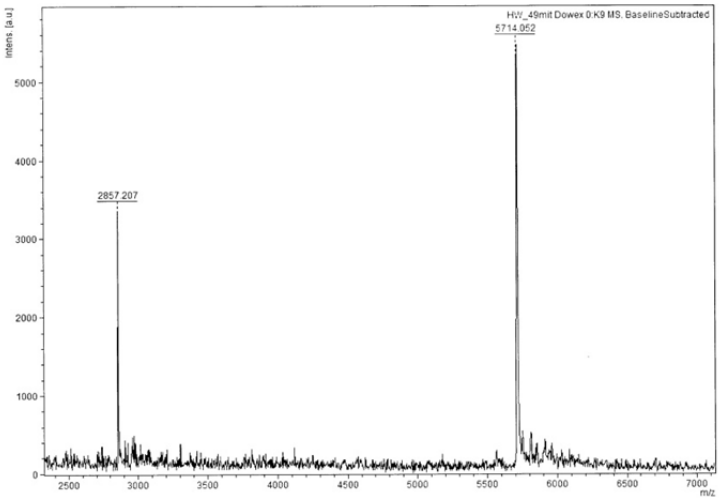
Scheme S77: MALDI-spectra of purified **DNA13**, calculated: 5586.4 Da, found: 5585.4 Da.



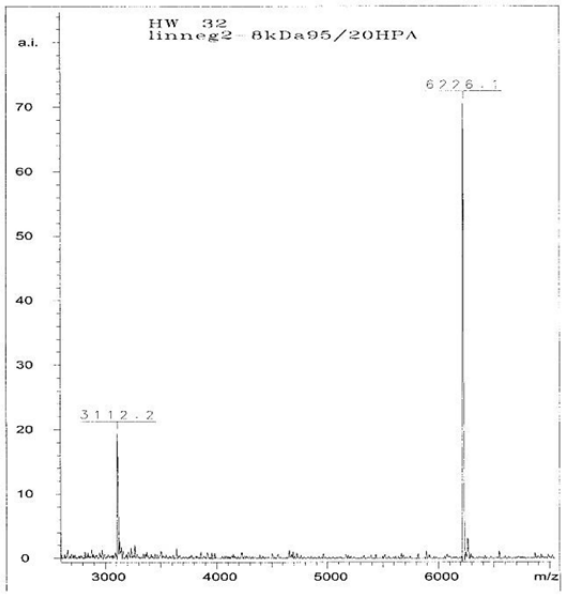
Scheme S78: MALDI-spectra of purified **DNA14**, calculated: 5712.6 Da, found: 5715.0 Da.



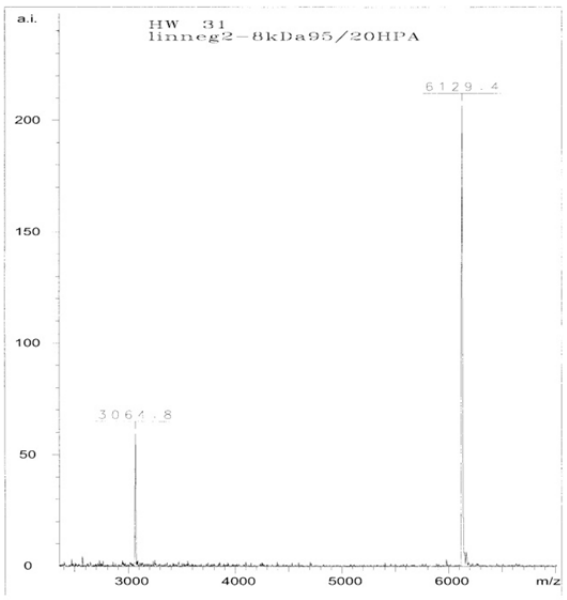
Scheme S79: MALDI-spectra of purified **DNA15**, calculated: 5586.4 Da, found: 5586.8 Da.



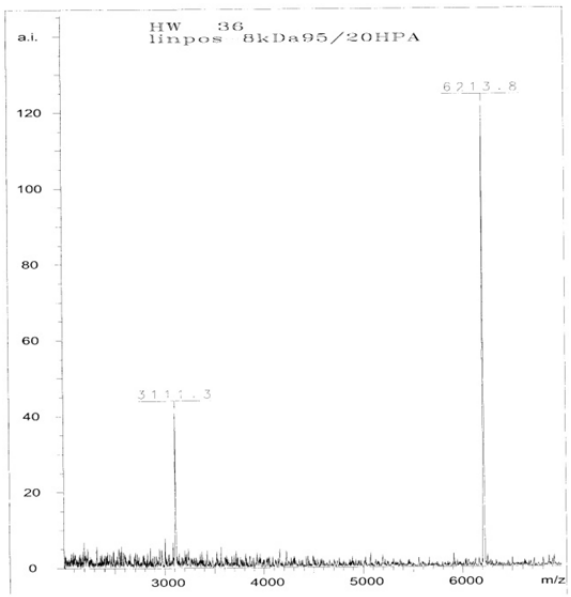
Scheme S80: MALDI-spectra of purified **DNA16**, calculated: 5712.6 Da, found: 5714.1 Da.



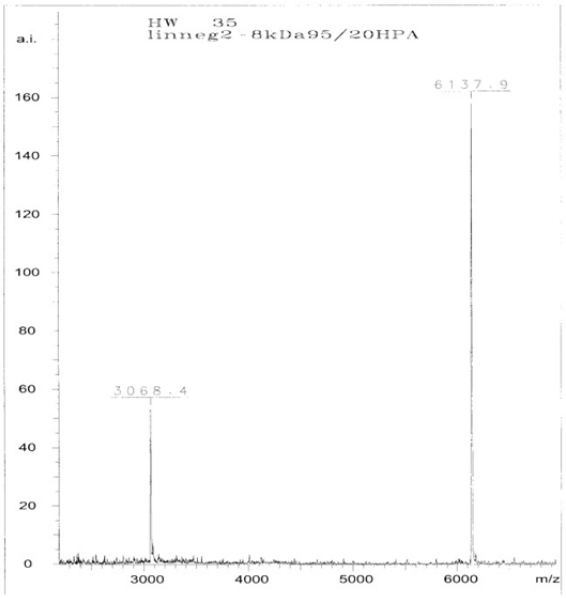
Scheme S81: MALDI-spectra of purified **DNA17**, calculated: 6223.2 Da, found: 6226.1 Da.



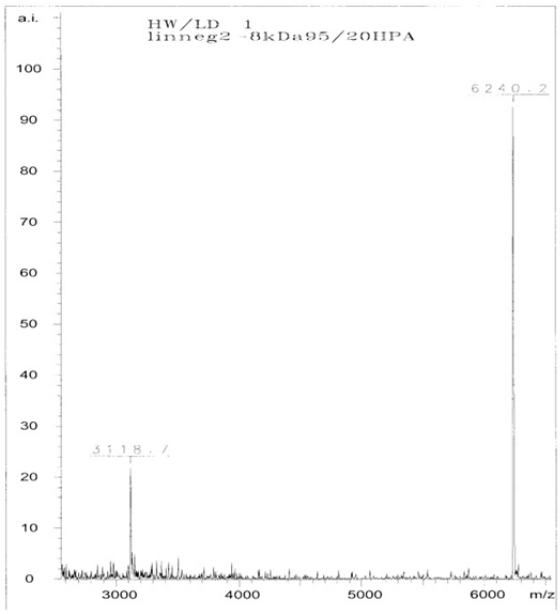
Scheme S82: MALDI-spectra of purified **DNA18**, calculated: 6125.2 Da, found: 6129.4 Da.



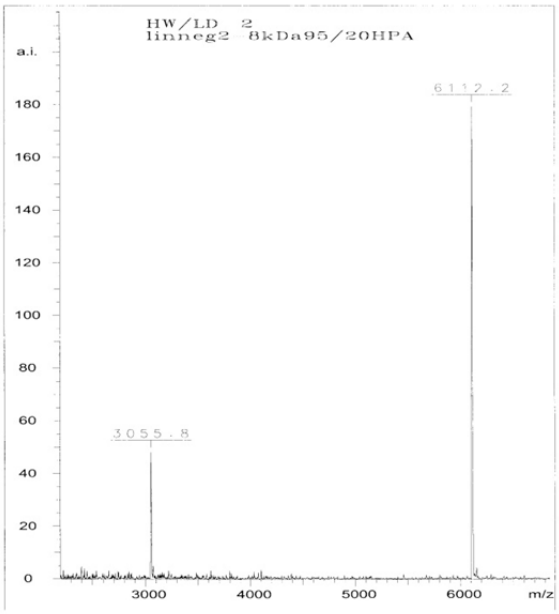
Scheme S83: MALDI-spectra of purified **DNA19**, calculated: 6214.2 Da, found: 6213.8 Da.



Scheme S84: MALDI-spectra of purified **DNA20**, calculated: 6134.2 Da, found: 6137.9 Da.

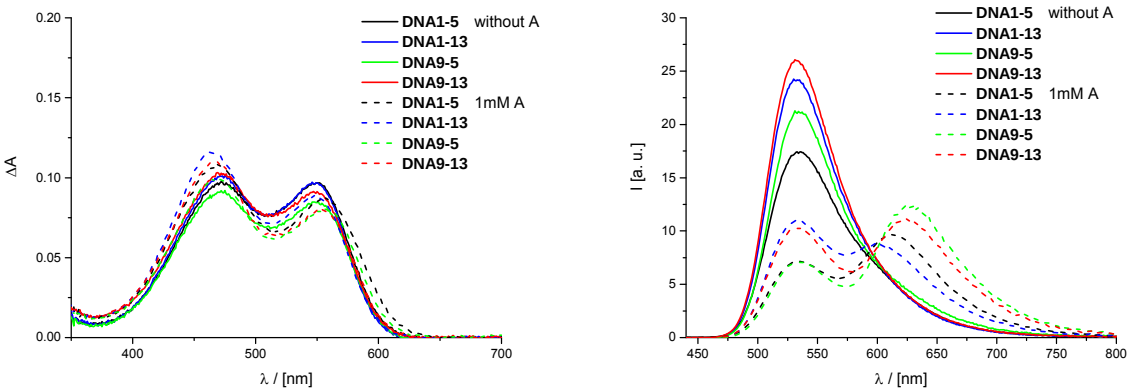


Scheme S85: MALDI-spectra of purified **DNA21**, calculated: 6238.2 Da, found: 6240.2 Da.

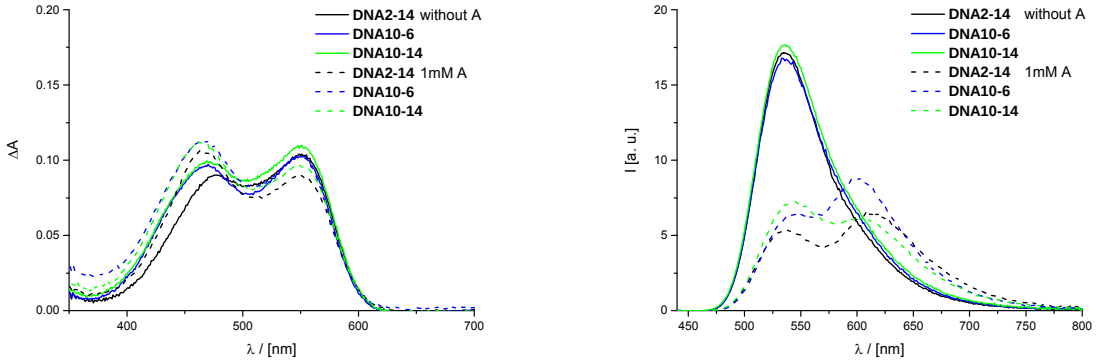


Scheme S86: MALDI-spectra of purified **DNA22**, calculated: 6109.2 Da, found: 6112.2 Da.

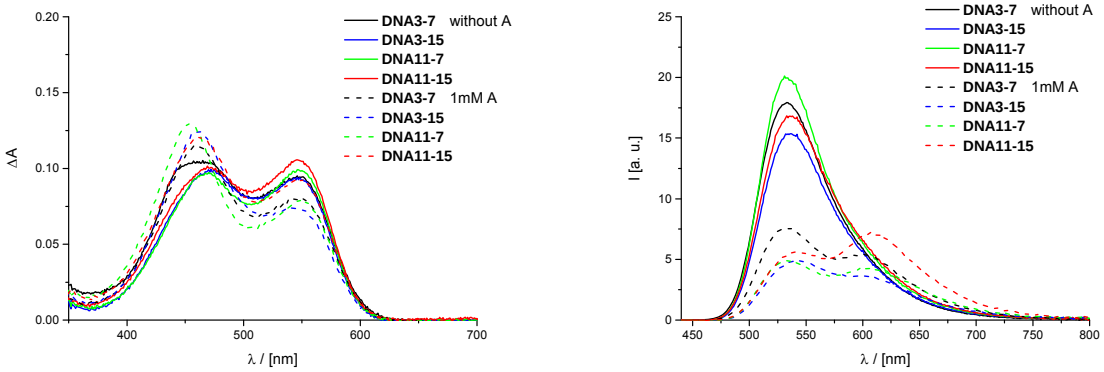
9. Additional spectroscopic data



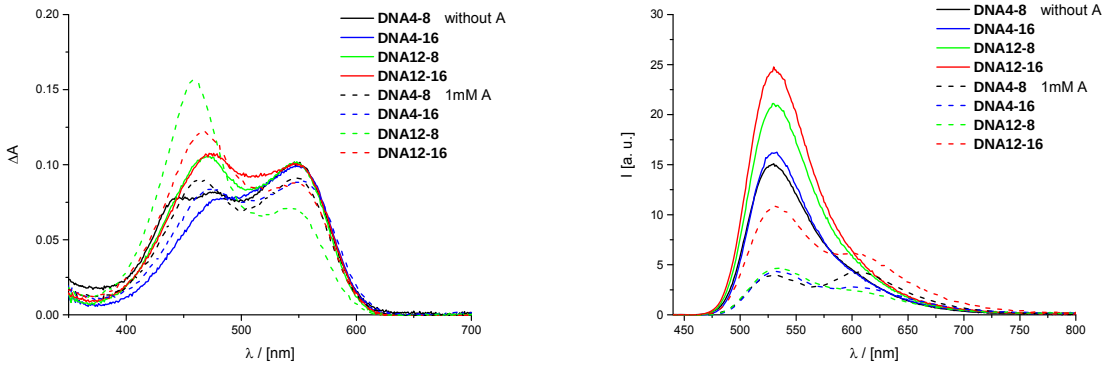
Scheme S87: Left: absorption spectra of DNA duplexes **DNA1-5**, **DNA1-13**, **DNA9-5** and **DNA9-13** without and with adenosine; right: fluorescence spectra



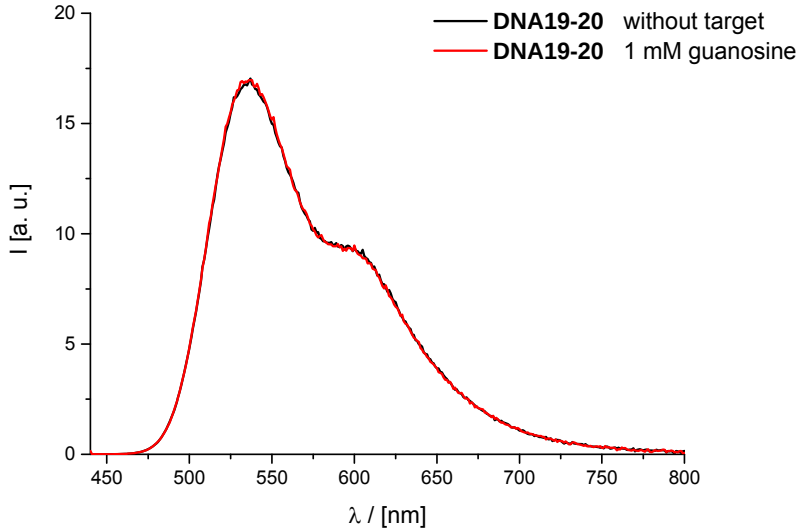
Scheme S88: right: absorption spectra left: emission spectra of **DNA2-14**, **DNA10-8**, **DNA2-14** with and without adenosine (A)



Scheme S89: right: absorption spectra left: emission spectra of **DNA3-7**, **DNA3-16**, **DNA11-7, DNA11-16** with and without adenosine (A)



Scheme S90: right: absorption spectra left: emission spectra of **DNA4-8**, **DNA4-16**, **DNA12-8, DNA12-18** with and without adenosine (A)



Scheme S91: emission spectra of **DNA19-20** without target and 1 mM guanosine.

10. References

[1] P. Kele, G. Mezo, D. Achatz, O. S., Wolfbeis *Angew. Chem. Int. Ed.* **2009**, *48*, 344-347.

[2] P. R. Bohländer, H.-A. Wagenknecht, *Org. Biomol. Chem.* **2013**, *11*, 7458-7462.